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(54) Title: PORCINE CELL INTERACTION PROTEINS

(57) Abstract

Antibodies to porcine P-selecting protein, porcine VCAM protein and porcine CD86 protein are useful for diagnosing human rejection of porcine xenotransplants and for improving xenotransplantation of porcine, cells, tissues and organs into human recipients.

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PORCINE CELL INTERACTION PROTEINS

FIELD OF THE INVENTION

This invention relates to xenotransplantation, and to the monitoring and modulation of the immune response to the xenotransplant. More specifically, the invention relates to the development of reagents and methods that will improve the ability to rapidly and specifically diagnose rejection of porcine xenotransplants by human patients. The invention also relates to compositions, including nucleic acid molecules, proteins (including antibodies), porcine cells, porcine tissues, and porcine organs, that will improve the outcome of the xenotransplantation of porcine cells, tissues, and organs into human recipients. To this end the invention provides a porcine P-selectin protein, a porcine VCAM protein, and a porcine CD86 protein, as well as the amino acid sequences of these proteins, the sequences of the cDNAs encoding these proteins, antibodies reactive with these proteins (but not with their human homologues), and methods for the use of these molecules.

BACKGROUND OF THE INVENTION

Xenotransplant Rejection: There is an ongoing shortage of human organs for transplant. This shortage has resulted in a long felt need for organs, and has resulted in attempts to develop xenotransplantation technology.

The primary non-primate candidate donor species for clinical xenotransplantation (e.g., the transplantation of non-human organs into human recipients) has been the pig. Swine provide an abundant supply of organs that are similar in size, anatomy, and physiology to their human counterparts (Auchincloss, 1988; Najarian, 1992; and Somervile and d'Apice, 1993). Transplantation of porcine pancreatic islets and of a pig liver into human patients has been reported, (Makowka et al., 1993; Satake et al., 1993; Tibell et al., 1993), but the outcomes of these transplants need to be improved. One improvement that is needed is better control (e.g., inhibition) of transplant rejection.

The rejection of transplanted organs may involve both an extremely rapid hyperacute rejection (HAR) phase and a slower cellular rejection phase. HAR of discordant (i.e., non-primate)

xenotransplants is initiated by preformed "natural" antibodies that bind to donor organ endothelium and activate complement attack by the recipient immune system (Dalmasso et al., 1992; and Tuso et al., 1993).

Activation of complement leads to the generation of fluid phase (C3a, C5a) and membrane bound (C3b and C5b-9, i.e., C5b, C6, C7, C8, and C9) proteins with chemotactic, procoagulant, proinflammatory, adhesive, and cytolytic properties (Muler-Eberhard, 1988). Immunohistological analysis of hyperacutely rejected xenotransplants reveals antibody deposition, complement fixation, and vascular thrombosis as well as neutrophil infiltration (Auchincloss, 1988; Mejia-Laguna et al., 1972; Najarian, 1992; Somervile and d'Apice, 1993; and Zehr et al., 1994).

While HAR is a major impediment to the xenotransplantation of vascularized organs, some discordantly xenotransplanted tissues (e.g., porcine pancreatic islets) do not appear to be rejected by this mechanism. Methods for the control of the HAR are also available. These include interference with the antibody antigen reactions responsible for initiating the HAR response, either by removing the antibodies from the circulation or by interfering with the expression of the antigens (see copending U.S. patent application Serial No. 08/214,580, entitled "Xenotransplantation Therapies" and filed by Mauro S. Sandrin and Ian F.C. McKenzie on March 15, 1994). Inhibition of complement attack on the xenotransplant may be accomplished by several means, including the use of complement inhibitors such as the 18kDa C5b-9 inhibitory protein and monoclonal antibodies against human C5b-9 proteins as taught in U.S. Patent No. 5,135,916, issued August 4, 1992.

In order to better understand the porcine xenograft rejection phenomenon, studies have been undertaken to investigate interactions between human white blood cells and porcine cells, particularly porcine aortic endothelial cells (PAEC). The role of neutrophils in the actual destruction of xenografts has not been well characterized, and the precise mechanism of complement independent neutrophil activation and adherence to xenograft endothelium are beginning to be understood.

Previous studies have shown that human complement component C3b (C3bi) deposited on PAEC mediates the binding of human neutrophils to the PAEC through interactions with the heterodimeric neutrophil cell surface receptor CD11b/CD18 (Vercellotti et al., 1991). Furthermore, blocking HAR by inhibition or depletion of complement results in decreased neutrophil infiltration and increased xenograft survival, providing additional evidence for the role of complement in mediating human neutrophil binding to porcine endothelium.

However, a significant neutrophil infiltrate into PAEC monolayers has been observed even in the absence of complement activation (Leventhal et al., 1993; and Pruitt et al., 1991). The development of such infiltrates is believed to play an important role in xenograft rejection, albeit not necessarily in hyperacute xenograft rejection. Means and methods allowing the control or elimination of such interactions are thus needed in order to make the transplantation of porcine cells, tissues, or organs into human recipients more practicable.

Cell interaction molecules: Numerous cell surface molecules serve to mediated cell-cell interactions such as cell adhesion and cell activation. These molecules include cell adhesion molecules such as P-selectin and VCAM, as well as "costimulatory" molecules, such as CD86 (B7-2) that are involved in the activation of certain cells of the immune system, e.g., T cells.

P-selectin: P-selectin (also known as CD62P, platelet activation-dependent granule external membrane protein - PADGEM, and granule membrane protein of molecular weight 140kDa - GMP-140) is a cytokine inducible cell adhesion molecule that is a glycoprotein found on alpha-granules of platelets and storage granules of endothelial cells, known as Weibel-Palade bodies (Bevilacqua and Nelson, 1993; Bonfanti et al., 1989; Collins et al., 1993) from whence it is released to the cell surface upon cell activation.

Structurally, P-selectin belongs to a family of adhesion molecules termed "selectins" that also includes E-selectin and L-selectin (see reviews in Lasky, 1992 and Bevilacqua and Nelson, 1993). These molecules are characterized by common structural features such as an amino-terminal lectin-like domain, an epidermal growth factor (EGF) domain, a discrete number of

complement repeat modules (approximately 60 amino acids each) similar to those found in certain complement binding proteins, a transmembrane domain, and a cytoplasmic tail (Dunlop et al., 1992).

P-selectin mediates the adhesion of various leukocytes (including neutrophils, monocytes, eosinophils, natural killer cells, and a subset of T cells) to activated platelets bound in the region of tissue injury, and to activated endothelium (Bevilacqua, et al., 1989; Carlos, et al., 1991; Graber, et al., 1990; Hakkert, et al., 1991; and Picker, et al., 1991; Shimuzu, et al., 1991). The importance of adhesive interactions with neutrophils is demonstrated by the observation that patients with an inherited defect in neutrophil adhesion exhibit neutrophilia and life-threatening bacterial infections of the classic leukocyte adhesion deficiency (LAD) syndrome (Carlos and Harlan, 1994; Lasky, 1992).

The expression of P-selectin is induced on human platelets and endothelial cells in response to thrombin generation, histamine generation, and the cytokines IL-1 and TNFa through transcriptional upregulation similar to that of E-selectin (Bevilacqua and Nelson, 1993; Carlos and Harlan, 1994). Phorbol esters, calcium ionophores, and complement proteins also activate P-selectin expression on endothelial cells (Collins et al., 1993; Hattori et al., 1989; Ishiwata et al., 1994).

Recent attempts to characterize the human leukocyte receptor for P-selectin have identified several different P-selectin ligands (Carlos and Harlan, 1994). These ligands contain sialic acid (sialyl Lewis x, or SLe^x) or other fucose-containing carbohydrate structures as a component mediating interaction with the P-selectin protein. Although SLe^x containing molecules seem to be higher affinity ligands, the number of these ligands and their precise specificity remains uncertain (Bevilacqua and Nelson, 1993; Carlos and Harlan, 1994).

Clinically, increased P-selectin expression on endothelium is associated with a variety of acute and chronic leukocyte-mediated inflammatory reactions. In addition to inflammation associated with graft rejection, leukocyte-mediated inflammatory reactions associated with increased P-selectin expression on endothelium include delayed type hypersensitivity, immune

complex-mediated lung injury, ischemic reperfusion injury, psoriasis, contact dermatitis, and arthritis, in addition to microcirculatory disorders such as thrombotic thrombocytopenic purpura (TTP) and hemolytic uremic syndrome (HUS) (Bevilacqua and Nelson, 1993; Carlos and Harlan, 1994; Ishiwata et al., 1994; Katayama et al., 1993; Mulligan et al., 1992).

During inflammatory reactions, P-selectin has been characterized as an adhesion molecule to mediate leukocyte "rolling" on the vessel wall where neutrophils emigrate from circulation to sites of injured tissue or graft tissue (Hattori et al., 1989). According to recent studies, increased C5b-9 complement protein stimulates platelets to secrete adhesion proteins for deposition of platelets at sites of inflammation (Collins et al., 1993; Hattori et al., 1989). Moreover, membrane deposition of C5b-9 proteins causes the release of very high molecular weight von Willebrand Factor multimers, which are accompanied by endothelial surface expression of an intracellular granule membrane protein, P-selectin. Thus, platelet activation regulates human responses to recognition of foreign tissue such that cytokine-induced expression of P-selectin by donor organ endothelium contributes to the binding and subsequent transmigration of inflammatory cells into the graft tissue and thereby plays an important role in acute cellular allograft rejection.

SOLUBLE P-SELECTIN

In normal humans, soluble P-selectin (sP-selectin) is known to exist in plasma at a concentration level of from 0.10 to 0.30 mg/ml (Carlos and Harlan, 1994; Dunlop et al., 1992; Ishiwata et al., 1994). The demonstration of sP-selectin in the blood would therefore be taken as evidence of either endothelial activation or platelet activation in diseases such as thrombotic and inflammatory diseases (Gearing and Newman, 1993; Dunlop et al., 1992). Gearing and Newman, 1993, review the levels of sP-selectin found in healthy and sick patients in various previous studies.

Elevated levels of sP-selectin have been found in patients with thrombotic thrombocytopenic purpura by a three-fold increase and hemolytic uremic syndrome by a two-fold increase (Gearing and Newman, 1993; Ushiyama et al., 1993). Similarly, sP-selectin was

detected in patients with circulatory disorders and adult respiratory distress syndrome (ARDS) with an increase of about 1 mg/ml.

Ushiyama et al., 1993 have cloned cDNAs encoding two recombinant forms of soluble P-selectin (sP-selectin). These soluble forms were characterized as having either a truncation after the 9th repeat or were lacking a transmembrane domain, encoded by exon 14, through alternative RNA splicing. Unlike the tetrameric P-selectin protein from platelet membranes, these soluble forms of P-selectin are monomeric. It is unknown where these monomers originate. However, studies suggest that soluble forms of P-selectin may have been produced by proteolytic cleavage of the protein or by shedding of the microvesicles containing the protein (Dunlop et al., 1992; Ushiyama et al., 1993). Other studies also suggest that sP-selectin were secreted as soluble forms from megakaryotes and vascular endothelial cells (Disdier et al., 1992; Ishiwata et al., 1994).

VCAM and CD86 are also cell adhesion molecules that are involved in the aggregation of various leukocytes at sites of inflammation. These molecules are also important mediators of inflammation, and are believed to be involved in xenograft rejection, albeit not necessarily in hyperacute xenograft rejection.

VCAM

Vascular cell adhesion molecule (VCAM) is an inducible transmembrane glycoprotein member of the immunoglobulin gene superfamily, expressed predominantly on endothelial cells (9-11). The interaction of VCAM with leukocytes is mediated by very late antigen-4 (VLA-4, a4b1), a b1 integrin molecule found on all leukocytes except neutrophils (12). VCAM expression is low or absent on resting endothelial cells in culture but can be induced by cytokines such as TNFa or IL-1 (9, 13-15). Thus, VCAM expression promotes a4 integrin-bearing leukocyte adhesion primarily to inflamed vascular endothelial cells (9, 15).

VCAM participates with intercellular adhesion molecule (ICAM) and endothelial-leukocyte adhesion molecule (ELAM) in the cellular recruitment, migration, and localization of inflammatory lymphocytes, monocytes, eosinophils and basophils to sites of tissue inflammation (8, 12, 14, 16). Recent in vitro and in vivo

studies performed under flow conditions have revealed that multiple receptor-ligand pairs can act sequentially and in an overlapping manner to effect leukocyte initial attachment, rolling, stable arrest and migration (17, 18). However, in an in vitro model that mimics microcirculatory flow conditions, a4b1-VCAM interactions were recently shown to be the predominant mechanism mediating the arrest of rolling T cells (17). The binding of VCAM to VLA-4 has been implicated in a variety of inflammatory and immune conditions involving leukocyte-endothelial cell adhesion, including both cardiac and renal allograft transplant rejection (18-23).

A role for VLA-4/VCAM interactions during the immune response to organ transfer has been shown by experiments in which treatment of experimental animals with mAbs to VCAM has delayed murine cardiac allograft rejection (20, 23). Anti-VLA-4 and anti-VCAM mAbs also have been shown to block migration of lymphocytes, monocytes and eosinophils into tissue, and to exhibit anti-inflammatory effects in animal models of experimental allergic encephalomyelitis (19-24).

SUMMARY OF THE INVENTION

In view of the foregoing state of the art, it is an object of this invention to prevent and/or treat xenograft rejection of porcine organs, tissues, or cells through modulation of P-selectin, VCAM, and/or CD86 mediated cell cell interactions, and to provide a means for diagnostic monitoring of xenotransplant rejection by specific measurement of the amount of porcine P-selectin and/or VCAM in the blood of the porcine xenotransplant recipient. It is a further object of the invention to provide antibody molecules that neither activate ("fix") complement, nor bind to the Fc receptor, particularly the FcRI receptor.

To achieve these and other goals, the invention provides:

1) Isolated porcine P-selectin and VCAM proteins.

2) Porcine P-selectin, VCAM, and CD86 genes, in the form of, for example, cDNA and genomic DNA molecules comprising porcine coding sequences.

3) A method for producing porcine P-selectin, VCAM, and CD86 by growing a recombinant host cell containing the gene of the invention (i.e., a nucleic acid molecule coding for porcine P-

selectin, VCAM, and/or CD86). The host cell is grown so that it expresses the porcine protein encoded by the gene of the invention and the expressed porcine protein is then isolated.

4) Anti porcine P-selectin antibodies that bind to porcine P-selectin, but not to human P-selectin; anti porcine VCAM antibodies that bind to porcine VCAM, but not to human VCAM; and anti porcine CD86 antibodies that bind to porcine CD86, but not to human CD86.

5) Therapeutic agents and methods for their use for the prevention and/or treatment of porcine xenograft rejection. These agents contain the porcine proteins of paragraph 1, immediately above, and/or the anti-porcine antibodies of paragraph 4, immediately above.

6) Agents for the diagnosis of porcine xenograft rejection based upon the anti-porcine P-selectin and anti-porcine VCAM antibodies of paragraph 4, immediately above.

7) Methods for disrupting the porcine genes of paragraph 1 in porcine cells, and the cell interaction molecule negative porcine cells generated via such methods.

8) Recombinant (chimeric and/or humanized) antibody molecules that react with porcine cell interaction proteins, but not with the analogous human cell interaction proteins.

9) Recombinant (chimeric and/or humanized) antibody molecules comprising the C1 and hinge regions of human IgG2 and the C2 and C3 regions of human IgG4, such antibodies being referred to hereinafter as "HuG2/G4 mAb".

The accompanying drawings, which are incorporated in and constitute part of the specification, illustrate the preferred embodiments of the invention, and together with the description, serve to explain the principles of the invention. It is to be understood, of course, that both the drawings and the description are explanatory only and are not restrictive of the invention.

BRIEF DESCRIPTION OF THE DRAWINGS

FIGURE 1. Adhesion of Ramos cells to TNFa-activated PAEC or COS-7 cells expressing pVCAM. Labeled Ramos cells were incubated for 30 min at 37°C with PAEC monolayers treated with 25 ng/ml recombinant human TNFa or with COS-7 monolayers transfected with APEX-1 (mock transfected) or pAPEX-1/pVCAM 72 h previously (no mAb). Specific adhesion of Ramos was analyzed by measuring dye

release of SDS cell lysates in a fluoremeter. Binding is expressed as the average Fluorescence units from three replicate wells with bars representing the standard error of the mean. The average background fluorescence from wells containing PAEC or COS-7 cells alone was ~130 units and was subtracted from the data. Inhibition of Ramos cell attachment to pVCAM expressing cells was carried out using an anti-human VLA-4 mAb (HP2/1; 10 ug/ml) or an isotype matched control mAb. The data presented are representative of three separate experiments.

FIGURE 2. spVCAM-His_6 fusion gene and protein. (A) Schematic of the putative structures of the full length pVCAM and truncated pVCAM. Six histidine residues and a stop codon and were inserted at the putative domain 7/transmembrane boundary. (B) Purification of spVCAM. spVCAM-His_6 protein was purified by adsorption and elution from Ni^{++} charged NTA resin as described in Materials and Methods, separated by SDS-PAGE under nonreducing conditions and stained with Coomassie Blue. The electrophoretic mobility of molecular mass standards is shown in kDa. Apparent differences in kDa are consistent with differential glycosylation of pVCAM-derived fragments, since potential N-glycosylation sites occur in domains 1, 2 and 3 (one site in each) and domain 6 (two sites) of pVCAM.

FIGURE 3. Adhesion of calcein-labeled Ramos cells to immobilized spVCAM. spVCAM was immobilized to plastic and assessed for the ability to support Ramos cell adhesion. (A) Concentration dependence of binding of Ramos cells to immobilized spVCAM. Adhesion of Ramos cells to the indicated concentrations of spVCAM is shown. spVCAM was immobilized to microtiter wells and 3×10^4 labeled Ramos cells in 0.1 ml RPMI/1640 medium containing 10% FBS were added to each well. Binding was quantitated after 30 min at 37°C. Background binding of Ramos cells to a negative control protein (BSA) was subtracted from the data. (B) Effect of mAb reactive with VLA-4 on binding of Ramos cells to immobilized spVCAM. Thirty thousand labeled Ramos cells were treated with anti-VLA-4 (HP2/1; 10 ug/ml) for 15 min at 37°C and added to microtiter wells precoated with a saturating concentration of

spVCAM (1 mg/well) or to BSA-coated control wells. Data are expressed as the average of triplicate wells. Experimental variation was less than 10%. Results presented are representative of three independent experiments.

FIGURE 4. Binding of Ramos cells to spVCAM in the continuous presence of mAbs to pVCAM. The indicated concentrations of anti-pVCAM mAb were added to microtiter wells precoated with spVCAM (1.0 mg/well) and incubated for 30 min at 37°C. Thirty thousand labeled Ramos cells in 0.1 ml RPMI/1640 medium containing 10% FBS were added to each microtiter well and binding examined after 30 min at 37°C. Binding is expressed as Fluorescence units. Representative data are shown from two experiments. Each value is a mean of triplicate wells.

FIGURE 5. Cell surface expression of VCAM on TNFa-activated HUVECs and PAEC. Cells were stained with anti-hVCAM (51-10C9) or anti pVCAM mAbs (2A2, 3F4, 5D11) followed by FITC goat-anti-mouse immunoglobulin and analyzed for VCAM expression by immunofluorecence and flow cytometry using a FACScan (Becton Dickinson Immunocytometry Systems). Data are displayed as histograms. The x-axis represents fluorescence and the y-axis represents the relative cell number. Background staining by secondary FITC-labeled antibody (SECONDARY) is indicated.

FIGURE 6. Epitope mapping of 2A2, 3F4 and 5D11 mAbs. Each anti-pVCAM mAb was assayed for the ability to bind to spVCAM captured on microtiter plates coated with either 2A2 or 3F4 F(ab')2 fragments. Detection of bound mAb was performed using peroxidase-conjugated goat anti-mouse IgG Fc. The background absorbance obtained in the absence of anti-pVCAM mAb was subtracted from all values. Results shown are the average of duplicate determinations.

FIGURE 7. Monoclonal antibody inhibition of Ramos and human peripheral T cell adhesion to TNFa-stimulated PAEC. Labeled Ramos or T cells were added to TNFa-stimulated PAEC monolayers in the presence or absence of the indicated mAb. Cell binding was quantitated in a 30 min adhesion assay. Each value is a mean of

triplicate wells with bars representing the standard error of the mean. Representative data are shown from three experiments using different blood donors. Each antibody was added at a final concentration of 10 ug/ml at the initiation of the assay.

FIGURE 8. Inhibition of Ramos cell binding to porcine aortic endothelial cells (PAEC). Cell adhesion assays were performed as described except the paec were stimulated with 1 µg/ml LPS for 16 hours prior to the assay. The binding reactions contained the indicated concentrations of either (A) 2A2 mAb, 2A2 $F(ab')_2$, or 2A2 Fab, or (B) 3F4 mAb, 3F4 $F(ab')_2$, or 3F4 Fab. Binding in the presence of inhibitor is defined as percent of binding found in the absence of inhibitor. The results demonstrate that only the bivalent inhibitors (2A2 mAb, 2A2 $F(ab')_2$, 3F4 mAb, 3F4 $F(ab')_2$) inhibited binding at concentrations of 3 to 10 µg/ml. Significantly higher concentrations of the monovalent 2A2 Fab or 3F4 Fab were required for inhibition of binding.

FIGURE 9. Sequences of the murine 2A2 and 3F4 variable regions,

FIGURE 10. Flow cytometry analysis of chimeric antibodies. (A) Murine antibodies 2A2 and 3F4 or purified chimeric antibodies (ch2A2 HuG4 and ch3F4 HuG4) were assayed for binding to 293-EBNA cells (293) or 293-EBNA cells expressing pVCAM (293/pVCAM). Cells were incubated with either no primary antibody (2°) or 10 µg/ml of the murine or chimeric antibodies. Bound antibody was detected using either FITC-conjugated goat anti-mouse IgG antibody or FITC-conjugated goat anti-human IgG antibody. (B) Murine 2A2 or 3F4 antibodies or the recombinant ch2A2 HuG4 and ch3F4 HuG4 were assayed for binding to PAEC stimulated with 1µg/ml LPS for approximately 16 hours. Results demonstrate identical staining using either the parental murine antibodies or the chimeric antibodies in both cases, indicating the appropriate variable regions had been cloned.

FIGURE 11. Inhibition of Ramos binding to PAEC. Binding experiments containing the indicated concentrations of antibody were performed as described in Figure 1. Results demonstrate the recombinant the 2A2 HuG4 and ch3F4 HuG4 inhibit binding as

potently as the murine 3F4 mAb. Neither a humanized antibody directed against human C5 (h5G1.1 CO12 HuG4 mAb) nor a murine antibody specific for human VCAM (anti-hVCAM) blocked binding of Ramos to PAEC.

FIGURE 12. Inhibition of Jurkat binding to PAEC. Binding experiments containing the indicated concentrations of antibody were performed as described in Figure 1 using calcein labeled Jurkat cells. Results demonstrate the recombinant the ch2A2 HuG4 and ch3F4 HuG4 inhibit binding as potently as the murine 3F4 mAb.

FIGURE 13. Inhibition of T-cell binding to PAEC. Binding experiments containing the indicated concentrations of inhibitor were performed as described in Figure 1 using calcein labeled purified human T-cells. Results demonstrate the recombinant the 2A2 HuG4 and ch3F4 HuG4 inhibit binding as potently as the murine 3F4 mAb.

FIGURE 14. Inhibition of U937 binding to PAEC. Binding experiments containing the indicated concentrations of antibody were performed as described in Figure 1 using calcein labeled U937 cells. Results demonstrate the recombinant ch3F4 HuG4 mAb does not inhibit binding, whereas the recombinant ch3F4 $F(ab')_2$ inhibits binding. This suggested that although the ch3F4 HuG4 mAb may have bound to the PAEC, U937 cells then adhered to the PAEC through interaction of the U937 cell FcRI receptor with the bound ch3F4 HuG4 mAb. To eliminate this interaction, chimeric antibodies containing the C1 and hinge region of human IgG2 and the C2 and C3 regions of human IgG4 were constructed (HuG2/G4 mAb). Flow cytometry demonstrated the resulting antibody does not bind to U937 cells. The ch3F4 HuG2/G4 mAb inhibited U937 binding to PAEC as potent as the ch3F4 HuG4 $F(ab')_2$.

FIGURE 15. Flow cytometry of HuG4 mAb and HuG2/G4 mAb binding to U937 cells. U937 cells were incubated with 10 µg/ml ch3F4 HuG4 mAb, ch3F4 HuG2/G4 mAb, ch2A2 HuG4 mAb, ch2A2 HuG2/G4 mAb, h5G1.1 CO12 HuG4 mAb, h5G1.1 CO12 HuG2/G4 mAb, or buffer. Bound antibody was detected using FITC-labeled goat anti-human IgG.

Results demonstrate that the HuG4 mAb bound to U937 cells whereas the HuG2/G4 mAb did not.

FIGURE 16. Assays of human neutrophil binding to PAEC.

FIGURE 17. Amino acid sequence of porcine P-selectin.

FIGURE 18. Soluble porcine P-selectin cell ELISAs.

FIGURE 19. FACS profiles of COS expression of porcine P-selectin.

FIGURE 20. Neutrophil binding to porcine P-selectin.

FIGURE 21. FACS analysis of porcine P-selectin expression by PAEC.

DETAILED DESCRIPTION OF PREFERRED EMBODIMENTS

The isolated nucleic acid molecules of the invention comprise sequences that are unique to the porcine genome. As used herein, the term "unique to the porcine genome" refers to sequences found in porcine-derived nucleic acid molecules that do not appear in published form as of the filing date of this application, e.g., they are not found in the cDNAs encoding the VCAM, P-selectin, or CD86 proteins of humans, cows, mice, or dogs.

The isolated nucleic acid molecules of the invention comprise sense sequences of contiguous nucleotides of the porcine sequences disclosed herein, for example in the figures. These sense sequences are unique to the porcine genome, and can be used as PCR primers or hybridization probes for the identification and/or isolation of the homologous porcine genes from genomic DNA. Antisense sequences of contiguous nucleotides complementary to such sense sequences are also required in order to practice PCR, and may also be used as hybridization probes. In order to be used for such purposes, the sequences of contiguous nucleotides must span a sufficient length. The minimum oligonucleotide length required for specific hybridization (i.e., hybridization under conditions requiring an essentially perfect match of complementary nucleotides wherein the sequence of the

probe can be expected to occur only once in the genome of the organism being probed) of both hybridization probes and PCR primers is well known in the art, and is discussed in, for example, Sambrook, et al, 1989, on pages 11.7-11.8. In practice, this span is at least 14 nucleotides, and, preferably, at least 18 nucleotides. Because at least 2 PCR primers are generally required to carry out a PCR reaction, the specificity of the PCR reaction is greater than that of each of the oligonucleotide primers used to drive the reaction.

Another isolated nucleic acid molecule of the invention is a cloned porcine genomic DNA molecule comprising a sequence of nucleotides unique to the porcine genome. This cloned molecule is characterized by hybridizing specifically to an isolated nucleic acid molecule as described in the preceding paragraph. Specific hybridization is used to clone this genomic DNA molecule. This cloning can be accomplished by several methods well known in the art such as by PCR using porcine genomic DNA templates, or by conventional screening of phage libraries of porcine genomic DNA, e.g., by plaque lift filter hybridization.

Certain of the isolated nucleic acid molecules of the invention are also useful as means to direct and/or modulate the expression of porcine cell interaction molecules in porcine cells, e.g., by altering the expression of any of the porcine P-selectin, VCAM, or CD86 genes. Such modulation may be accomplished by several means well known in the art. Modulation, specifically inhibition, of the expression of any particular gene may be accomplished by the use of antisense nucleic acid molecules or DNA constructions specially engineered to allow gene inactivation as described below for antisense RNAs, antisense oligonucleotides, and gene knockout constructions. For example, for the inhibition of the porcine VCAM, the antisense nucleic acid molecules or DNA constructions will comprise nucleic acid sequences of the VCAM nucleic acid molecules of the invention.

Antisense RNAs can be used to specifically inhibit gene expression (see, for example, Eguchi, et al., 1991). Such nucleic acid molecules can be expressed by recombinant transcription units engineered for expression in porcine cells. Such transcription units can be introduced as transgenes into porcine

cells, and, when introduced into porcine embryos or embryonic stem cells can be used to generate transgenic pigs.

Antisense nucleic acid molecules in the form of oligonucleotides (including oligonuclotide analogs) and derivatives thereof can also be used to specifically inhibit gene expression, as described, for example, in Cohen, 1989. As described therein, antisense oligonucleotides can be designed and used to inhibit expression of specific genes (Cohen, 1989, pp. 1-6, 53-77).

Such antisense oligonucleotides can be in the form of oligonucleotide analogs, for example, phosphorothioate analogs (Cohen, 1989, pp. 97-117), non-ionic analogs (Cohen, 1989, pp. 79-95), and a-oligodeoxynucleotide analogs (Cohen, 1989, pp. 119-136). Derivatives of oligonucleotides that can be used to inhibit gene expression include oligonucleotides covalently linked to intercalating agents or to nucleic acid-cleaving agents (Cohen, 1989, pp. 137-172), and oligonucleotides linked to reactive groups (Cohen, 1989, pp. 173-196). Oligonucleotides and derivatives designed to recognize double-helical DNA by triple-helix formation (Cohen, 1989, pp. 197-210) may also be used to specifically inhibit gene expression.

All of the oligonucleotides and derivatives described above are used by adding them to the fluids bathing the cells in which specific inhibition of gene expression in accordance with the present invention is desired.

Another method by which the expression of specific genes can be inhibited is by genetic manipulations referred to in the art as "gene disruption" or "gene knockout." Gene knockout is a method of genetic manipulation via homologous recombination that has long been carried out in microorganisms, but has only been practiced in mammalian cells within the past decade. These techniques allow for the use of specially designed DNA molecules (gene knockout constructions) to achieve targeted inactivation (knockout) of a particular gene upon introduction of the construction into a cell. The practice of mammalian gene knockout, including the design of gene knockout constructions and the detection and selection of successfully altered mammalian cells, is discussed in numerous publications, including Thomas, et al., 1986; Thomas, et al., 1987; Jasin and Berg, 1988;

Mansour, et al., 1988; Brinster, et al., 1989; Capecchi, 1989; Frohman and Martin, 1989; Hasty, et al., 1991; Jeannotte, et al., 1991; and Mortensen, et al., 1992.

Gene knockouts and gene replacements can be achieved in mammalian zygotes through microinjection techniques well known in the art (Brinster, et al., 1989). The introduction of the DNA constructions used to effect gene knockouts into cultured cells is a more common route to the production of knockout cells, tissues, and organs. In those cases where knockout tissues or organs are desired, cultured embryonic stem cells provide a means to introduce the DNA constructions into cells in culture and to generate transgenic animals derived from such engineered cells. Such animals can also be obtained from knockout transgenic zygotes obtained by microinjection as described above.

Thus, in accordance with certain aspects of the invention, the nucleic acid molecules of the present invention are used to generate engineered transgenic animals using techniques known in the art. These techniques include, but are not limited to, microinjection, e.g., of nuclei or pronuclei, electroporation of ova or zygotes, nuclear transplantation, and/or the stable transfection or transduction of embryonic stem cells.

The most well known method for making transgenic animals is that used to produce transgenic mice by superovulation of a donor female, surgical removal of the egg, injection of the transgene transcription unit into the pronuclei of the embryo, and introduction of the transgenic embryo into the reproductive tract of a pseudopregnant host mother, usually of the same species. See Brinster, et al., 1985, Hogan, et al., 1986, Robertson 1987, Pedersen, et al., 1990.

The use of this method to make transgenic livestock is also widely practiced by those of skill in the art. As an example, transgenic swine are routinely produced by the microinjection of nucleic acid molecules into pig embryos. See, for example, PCT Publication No. WO92/11757. In brief, this procedure may, for example, be performed as follows. First, the nucleic acid molecules are gel isolated and extensively purified, for example, through an ELUTIP column (Schleicher & Schuell, Keene, NH), dialyzed against pyrogen free injection buffer (10mM Tris, pH7.4

+ 0.1mM EDTA in pyrogen free water), and used for embryo injection.

Embryos are recovered from the oviduct of a hormonally synchronized, ovulation induced sow, preferably at the pronuclear stage. They are placed into a 1.5 ml microfuge tube containing approximately 0.5 ml of embryo transfer media (phosphate buffered saline with 10% fetal calf serum). These are centrifuged for 12 minutes at 16,000 x g in a microcentrifuge. Embryos are removed from the microfuge tube with a drawn and polished Pasteur pipette and placed into a 35 mm petri dish for examination. If the cytoplasm is still opaque with lipid such that the pronuclei are not clearly visible, the embryos are centrifuged again for an additional 15 minutes.

Embryos to be microinjected are placed into a drop of media (approximately 100 µl) in the center of the lid of a 100 mm petri dish. Silicone oil is used to cover this drop and to fill the lid to prevent the medium from evaporating. The petri dish lid containing the embryos is set onto an inverted microscope equipped with both a heated stage (37.5-38°C) and Hoffman modulation contrast optics (200X final magnification). A finely drawn and polished micropipette is used to stabilize the embryos while about 1-2 picoliters of injection buffer containing approximately 200-500 copies of the purified transgene transcription unit is delivered into the nucleus, preferably the male pronucleus, with another finely drawn and polished micropipette. Embryos surviving the microinjection process as judged by morphological observation are loaded into a polypropylene tube (2 mm ID) for transfer into the recipient pseudopregnant sow.

Offspring are tested for the presence of the transgene by isolating genomic DNA, e.g., from tissue removed from the tail of each piglet, and subjecting about 5 micrograms of this genomic DNA to nucleic acid hybridization analysis with a transgene specific probe.

Another commonly used technique for generating transgenic animals involves the genetic manipulation of embryonic stem cells (ES cells) as described in PCT Patent Publication No. WO 93/02188 and Robertson, 1987. In accordance with this technique, ES cells are grown as described in, for example, Robertson, 1987,

and in U.S. Patent No. 5,166,065 to Williams et al. Genetic material is introduced into the embryonic stem cells by, for example, electroporation according, for example, to the method of McMahon, et al., 1990, or by transduction with a retroviral vector according, for example, to the method of Robertson, et al., 1986, or by any of the various techniques described by Lovell-Badge, 1987.

Chimeric animals are generated as described, for example, in Bradley, 1987. Briefly, genetically modified ES cells are introduced into blastocysts and the modified blastocysts are then implanted in pseudo-pregnant female animals. Chimeras are selected from the offspring, for example by the observation of mosaic coat coloration resulting from differences in the strain used to prepare the ES cells and the strain used to prepare the blastocysts, and are bred to produce non-chimeric transgenic animals.

Other methods for the production of transgenic animals are disclosed in U.S. Patent No. 5,032,407 to Wagner et al., and PCT Publication No. WO90/08832.

The practice of gene knockout in embryonic stem cells, and the generation of engineered animals from such cells, is discussed in numerous publications, including Thomas, et al., 1987; Robertson, 1987; Mansour, et al., 1988; Capecchi, 1989; Frohman and Martin, 1989; Hasty, et al., 1991; Jeannotte, et al., 1991; Mortensen, et al., 1992; Thomas, et al., 1992; and PCT Patent Publication No. WO 93/02188.

Among other applications, transgenic pigs prepared in accordance with the invention are useful as model systems for testing the xenotransplantation of their engineered cells, tissues, or organs and as sources of engineered cells, tissues, or organs for xenotransplantation. The lack of expression of porcine porcine cell interaction proteins on the endothelial cells of the transgenic pigs will provide enhanced protection from rejection following xenotransplantation of those cells, or of tissues and organs containing those cells, into recipient animals, e.g., humans. In addition to their use in producing tissues, and organs for transplantation, the nucleic acid molecules of the invention can also be used to directly engineer

cultured porcine endothelial cells for subsequent use in transplantation.

The nucleic acid molecules of the invention can also be used to express porcine cell interaction proteins for subsequent purification and use. Recombinant DNA methods for the production of recombinant proteins are well known in the art, as are methods for the purification of such proteins (see, for example, Ausubel, et al., 1992; Goeddel, 1990; Harris and Angal, 1989; and Deutscher, 1990).

Preferred uses of such proteins include the use of porcine cell interaction proteins as immunogens for the purpose of raising anti porcine cell interaction protein antibodies, or as an antigen for use in immunoassays to detect soluble porcine cell interaction proteins as markers of inflammation in primate recipients of porcine xenografts. See, for example, below under "ELISA screen for anti-porcine VCAM antibodies".

The present invention provides recombinant expression vectors which include synthetic or cDNA-derived DNA fragments encoding porcine cell interaction proteins. The nucleotide sequences coding for porcine cell interaction proteins can be inserted into an appropriate expression vector, i.e., a vector that contains the necessary elements for the transcription and translation of the inserted protein-coding sequence. The necessary transcriptional and translational signals can also be supplied by the native gene and/or its flanking regions. A variety of host vector systems may be utilized to express the protein-coding sequence. These include, but are not limited to, mammalian cell systems infected with virus (e.g., vaccinia virus, adenovirus, retroviruses, etc.); mammalian cell systems transfected with plasmids; insect cell systems infected with virus (e.g., baculovirus); microorganisms such as yeast containing yeast expression vectors, or bacteria transformed with bacteriophage DNA, plasmid DNA, or cosmid DNA (see, for example, Goeddel, 1990).

Useful expression vectors for bacterial use can comprise a selectable marker and bacterial origin of replication derived from commercially available plasmids comprising genetic elements of the well-known cloning vector pBR322 (American Type Culture Collection, 12301 Parklawn Drive, Rockville, Maryland 20852,

United States of America; ATCC Accession No. 37017). These pBR322 "backbone sections," or functionally equivalent sequences, are combined with an appropriate promoter and the structural gene to be expressed. Promoters commonly used in recombinant microbial expression vectors include, but are not limited to, the lactose promoter system (Chang, et al., 1978), the tryptophan (trp) promoter (Goeddel, et al., 1980) and the tac promoter, or a fusion between the tac and trp promoters referred to as the trc promoter (Maniatis, 1982). Preferred bacterial expression vectors include, but are not limited to, vector pSE420 (Invitrogen Corporation, San Diego, California). This vector harbors the trc promoter, the lacO operon, an anti-terminator sequence, the g10 ribosome binding sequence, a translation terminator sequence, the lacIq repressor, the ColE1 origin of replication, and the ampicillin resistance gene.

Recombinant porcine cell interaction proteins may also be expressed in fungal hosts, preferably yeast of the Saccharomyces genus such as S. cerevisiae. Fungi of other genera such as Aspergillus, Pichia or Kluyveromyces may also be employed. Fungal vectors will generally contain an origin of replication from the 2 μm yeast plasmid or another autonomously replicating sequence (ARS), a promoter, DNA encoding a porcine cell interaction molecule, sequences directing polyadenylation and transcription termination, and a selectable marker gene. Preferably, fungal vectors will include an origin of replication and selectable markers permitting transformation of both E. coli and fungi.

Suitable promoter systems in fungi include the promoters for metallothionein, 3-phosphoglycerate kinase, or other glycolytic enzymes such as enolase, hexokinase, pyruvate kinase, glucokinase, the glucose-repressible alcohol dehydrogenase promoter (ADH2), the constitutive promoter from the alcohol dehydrogenase gene, ADH1, and others. See, for example, Schena, et al. 1991. Secretion signals, such as those directing the secretion of yeast a-factor or yeast invertase, can be incorporated into the fungal vector to promote secretion of a soluble porcine cell interaction proteins into the fungal growth medium. See Moir, et al., 1991.

Preferred fungal expression vectors can be assembled using DNA sequences from pBR322 for selection and replication in bacteria, and fungal DNA sequences, including the ADH1 promoter and the alcohol dehydrogenase ADH1 termination sequence, as found in vector pAAH5 (Ammerer, 1983). The ADH1 promoter is effective in yeast in that ADH1 mRNA is estimated to be 1 - 2% of total poly(A) RNA.

Various mammalian or insect cell culture systems can be employed to express recombinant porcine cell interaction proteins. Suitable baculovirus systems for production of heterologous proteins in insect cells are reviewed by Luckow, et al., 1988. Examples of suitable mammalian host cell lines include the COS cell of monkey kidney origin, mouse L cells, murine C127 mammary epithelial cells, mouse Balb/3T3 cells, Chinese hamster ovary cells (CHO), human 293 EBNA and HeLa cells, myeloma, and baby hamster kidney (BHK) cells. Mammalian expression vectors may comprise non-transcribed elements such as origin of replication, a suitable promoter and enhancer linked to the porcine cell interaction protein gene to be expressed, and other 5' or 3' flanking sequences such as ribosome binding sites, a polyadenylation sequence, splice donor and acceptor sites, and transcriptional termination sequences.

The transcriptional and translational control sequences in mammalian expression vector systems to be used in transforming vertebrate cells may be provided by viral sources. For example, commonly used promoters and enhancers are derived from Polyoma virus, Adenovirus, Simian Virus 40 (SV40), and human cytomegalovirus, including the cytomegalovirus immediate-early gene 1 promoter and enhancer (CMV).

Particularly preferred eukaryotic vectors for the expression of porcine cell interaction proteins are pAPEX-1 and pAPEX-3, as described below. A particularly preferred host cell for the expression of inserts in the pAPEX-3 vector is the human 293 EBNA cell line (Invitrogen, San Diego, CA).

Another preferred eukaryotic vector for the expression of porcine cell interaction proteins is pcDNAI/Amp (Invitrogen Corporation, San Diego, California). The pcDNAI/Amp expression vector contains the human cytomegalovirus immediate-early gene I promoter and enhancer elements, the Simian Virus 40 (SV40)

consensus intron donor and acceptor splice sequences, and the SV40 consensus polyadenylation signal. This vector also contains an SV40 origin of replication that allows for episomal amplification in cells (e.g., COS cells, MOP8 cells, etc.) transformed with SV40 large T antigen, and an ampicillin resistance gene for propagation and selection in bacterial hosts.

Purified porcine cell interaction proteins are prepared by culturing suitable host/vector systems to express the recombinant translation products of the nucleic acid molecules of the present invention, which are then purified from the culture media or cell extracts of the host system, e.g., the bacteria, insect cells, fungal, or mammalian cells. Fermentation of fungi or mammalian cells that express soluble porcine cell interaction proteins containing a histidine tag sequence (comprising a string of at least 5 histidine residues in a row) as a secreted product greatly simplifies purification. Such a histidine tag sequence enables binding under specific conditions to metals such as nickel, and thereby to nickel columns for purification.

In general terms, the purification of recombinant porcine cell interaction proteins is performed using a suitable set of concentration, fractionation, and chromatography steps well known in the art (see, for example, Deutscher, 1990; and Harris and Angal, 1989). For recombinant porcine cell interaction proteins requiring correct disulfide bond formation for full biological activity, denaturation of the purified protein followed by chemical-mediated refolding under reducing conditions can be done to promote proper disulfide interactions.

Porcine cell interaction proteins purified from bodily fluids of transgenic animals engineered to produce the porcine cell interaction proteins of the invention are also within the scope of the invention, as are porcine cell interaction proteins that are produced in part or entirely by chemical synthesis.

Porcine cell interaction proteins synthesized in recombinant culture and subsequently purified may be characterized by the presence of contaminating components. These components may include proteins or other molecules in amounts and of a character which depend on the production and purification processes. These components will ordinarily be of viral, prokaryotic, eukaryotic, or synthetic origin, and preferably are present in innocuous

contaminant quantities, on the order of less than about 1% by weight. Recombinant cell culture, however, enables the production of porcine cell interaction proteins relatively free of other proteins that may normally be associated with the proteins as found in nature.

As discussed above, certain aspects of the present invention relates to the use of anti porcine cell interaction protein antibodies or soluble cell interaction proteins (collectively referred to hereinafter as "therapeutic porcine cell interaction agents") in treating patients suffering from xenotransplant rejection. The therapeutic porcine cell interaction agents are used in an amount effective to achieve blood concentrations equivalent to in vitro concentrations that substantially reduce (e.g., reduce by at least about 50%) the binding of human test cells expressing the human cell interaction protein binding ligand, such as PBLs, neutrophils, and HL-60 cells, to cells expressing porcine cell interaction proteins, such as TNFa treated porcine endothelial cells. Reduction of the binding of human test cells to cells expressing porcine cell interaction proteins can be measured by methods well known in the art such as, for example, by the assay described below under the heading "assays for neutrophil / HL-60 binding to PAEC".

To achieve the desired reductions in binding, the therapeutic porcine cell interaction agents can be administered in a variety of unit dosage forms. The dose will vary according to the particular agent. For example, different antibodies may have different masses and/or affinities, and thus require different dosage levels. Antibodies prepared as Fab' or $F(ab')_2$ fragments will also require differing dosages than the equivalent intact immunoglobulins, as they are of considerably smaller mass than intact immunoglobulins, and thus require lower dosages to reach the same molar levels in the patient's blood.

The dose will also vary depending on the manner of administration, the particular symptoms of the patient being treated, the overall health, condition, size, and age of the patient, and the judgment of the prescribing physician. Dosage levels of the therapeutic porcine cell interaction agents for human subjects are generally between about 1 mg per kg and about 100 mg per kg per patient per treatment, and preferably between

about 5 mg per kg and about 50 mg per kg per patient per treatment. In terms of plasma concentrations, the therapeutic porcine cell interaction agent concentrations are preferably in the range from about 25 µg/ml to about 500 µg/ml. See, also, Kung et al., 1993.

Subject to the judgment of the physician, a typical therapeutic treatment includes a series of doses, which will usually be administered concurrently with the monitoring of clinical endpoints such as xenotransplant biopsies, or measures of organ function, such as, for example, for xenotransplanted kidneys, BUN levels, proteinuria levels, etc., with the dosage levels adjusted as needed to achieve the desired clinical outcome.

The therapeutic porcine cell interaction agents of the present invention can be used in therapeutic compositions to treat episodes of xenograft rejection. Such treatment will result in the reduction of the severity of the rejection episode. For such application, purified therapeutic porcine cell interaction agents can be administered to a patient, e.g., a human, in a variety of ways. Thus, therapeutic porcine cell interaction agents can be given by bolus injection, continuous infusion, sustained release from implants, or other suitable techniques.

Formulations suitable for injection are found in Remington's Pharmaceutical Sciences, Mack Publishing Company, Philadelphia, PA, 17th ed. (1985). Such formulations must be sterile and non-pyrogenic, and generally will include purified therapeutic porcine cell interaction agents in conjunction with a pharmaceutically effective carrier, such as saline, buffered (e.g., phosphate buffered) saline, Hank's solution, Ringer's solution, dextrose/saline, glucose solutions, and the like. The formulations may contain pharmaceutically acceptable auxiliary substances as required, such as, tonicity adjusting agents, wetting agents, bactericidal agents, preservatives, stabilizers, and the like.

In one preferred embodiment, the therapeutic porcine cell interaction agent is formulated as a lyophilizate using appropriate excipient solutions (e.g., sucrose, albumin) as diluents. The amount and frequency of administration will

depend, of course, on such factors as the nature and severity of the rejection episode being treated, the desired response, the condition of the patient, and so forth.

The formulations of the invention can be distributed as articles of manufacture comprising packaging material and the therapeutic porcine cell interaction agents. The packaging material will include a label which indicates that the formulation is for use in the treatment of porcine xenotransplant rejection.

Hybridomas producing the monoclonal antibodies of the invention, i.e., monoclonal antibodies reactive with porcine cell interaction proteins, but not with human cell interaction proteins, can be obtained using purified porcine cell interaction proteins as immunogens followed by screening. Such screening is carried out to identify hybridomas producing antibodies with the desired properties, and can be carried out using appropriate immunoassays. Examples of appropriate immunoassays are the ELISA described below and in copending U.S. patent application serial No.: 08/252,493, filed June 1, 1994, which is incorporated herein by reference. A simple modification of this ELISA (i.e., substituting soluble human cell interaction proteins for soluble porcine cell interaction proteins) can be used to identify those of the hybridomas producing antibodies that bind to porcine cell interaction proteins in which the antibodies do not bind to human cell interaction proteins.

General methods for the immunization of animals (in this case with isolated porcine cell interaction proteins), isolation of antibody producing cells, fusion of such cells with immortal cells (e.g., myeloma cells) to generate hybridomas secreting monoclonal antibodies, screening of hybridoma supernatants for reactivity and/or lack of reactivity of secreted monoclonal antibodies with particular antigens (in this case reactivity with a porcine cell interaction protein but not with the corresponding human cell interaction protein), the preparation of quantities of such antibodies in hybridoma supernatants or ascites fluids, and for the purification and storage of such monoclonal antibodies, can be found in numerous publications. These include: Coligan, et al., eds. Current Protocols In Immunology, John Wiley & Sons, New York, 1992; Harlow and Lane, Antibodies, A Laboratory Manual,

Cold Spring Harbor Laboratory, New York, 1988; Liddell and Cryer, A Practical Guide To Monoclonal Antibodies. John Wiley & Sons, Chichester, West Sussex, England, 1991; Montz, et al., 1990; Wurzner, et al., 1991; and Mollnes, et al., 1988.

The present invention also includes porcine cell interaction proteins and anti porcine cell interaction protein antibodies with or without associated native patterns of glycosylation. For example, expressing proteins recombinantly in bacteria such as E. coli provides non-glycosylated molecules, while expressing porcine cell interaction proteins or anti porcine cell interaction protein antibodies in mammalian cells can provide glycosylated molecules.

As used herein, the term "antibodies" refers to immunoglobulins produced in vivo, as well as those produced in vitro by a hybridoma, and antigen binding fragments (e.g., Fab' preparations) of such immunoglobulins, as well as to recombinantly expressed antigen binding proteins, including immunoglobulins, chimeric immunoglobulins, "humanized" immunoglobulins, antigen binding fragments of such immunoglobulins, single chain antibodies, and other recombinant proteins containing antigen binding domains derived from immunoglobulins. Publications describing methods for the preparation of such antibodies, in addition to those listed immediately above, include: Reichmann, et al., 1988; Winter and Milstein, 1991; Clackson, et al., 1991; Morrison, 1992; Haber, 1992; and Rodrigues, et al., 1993.

Diagnostic use of the anti porcine cell interaction protein antibodies of the invention can be carried out by assaying the patient's blood for levels of one or more porcine cell interaction proteins. Assays for porcine cell interaction protein levels may be by RIA, ELISA, or other suitable immunoassay using the anti porcine cell interaction protein antibodies of the invention. General methods for performing such assays are set forth in Coligan, et al., 1992. Blood porcine cell interaction protein levels must be monitored at regular intervals, e.g., daily or weekly, and changes in such levels recorded. Any distinct increase in porcine cell interaction protein levels in the patient's blood is an indication that the

porcine tissue is becoming inflamed, and may indicate the onset of a rejection episode.

An alternative test for rejection, (or a test providing confirmation of the occurrence of rejection as indicated by measurement of soluble cell interaction protein levels) may be obtained by monitoring porcine organ function or by biopsy and histopathological examination of the porcine organ. Such examination will be carried out in order to detect the typical manifestations of transplant rejection, e.g., cellular infiltrates, inflammation, and necrosis. In accordance with the invention, the histopathological examination of xenotransplanted organ biopsy tissues will also include the use of certain of the antibodies of the invention to detect the levels of expression of one or more porcine cell interaction proteins on the surfaces of the cells of the biopsied tissues of the xenotransplanted organ. High levels of such expression (compared to levels on non-transplanted control tissue samples) are indicative of xenotransplant rejection.

Although specific embodiments of the invention are described and illustrated herein, it is to be understood that modifications can be made without departing from the invention's spirit and scope.

For example, the nucleotide sequences of the porcine cell interaction protein-encoding nucleic acid molecules of the invention may be modified by creating nucleic acid mutations which do not significantly change the encoded amino acid sequences. Such mutations include third nucleotide changes in degenerate codons (and other "silent" mutations that do not change the encoded amino acid sequence).

Other such mutations within the scope of the invention and considered as equivalents of the specific embodiments set forth herein include those which result in a highly conservative amino acid substitution for an encoded amino acid while leaving the leucocyte binding (or other cell binding) characteristics of the porcine cell interaction proteins essentially unchanged. Such silent or highly conservative mutations are included within the scope of the invention.

Also included are:

1) Nucleotide and amino acid sequences comprising changes that are found as naturally occurring allelic variants of the porcine cell interaction protein genes;

2) Sequences which have been truncated so as to only encode the mature porcine cell interaction protein polypeptides, i.e., a porcine cell interaction polypeptide without the amino terminal leader sequence that directs the protein to its typical transmembrane orientation in the cell;

3) Sequences in which the cell interaction protein amino terminal leader sequences have been altered, e.g., substituted with a different leader;

4) Sequences in which a peptide "tag" sequence has been inserted or added on to enable the ready identification and/or purification of recombinant proteins. Such tags include the FLAG epitope (which enables specific binding to anti-FLAG antibodies) and a histidine tag sequence, as described above;

5) Sequences that have been altered to produce a soluble porcine cell interaction protein by, for example, truncation.

Without intending to limit it in any manner, the present invention will be more fully described by the following examples. Materials and methods used in various of the examples are as follows.

MATERIALS AND METHODS

Materials: A monoclonal antibody to human LFA-1 (clone 25.3) was obtained from AMAC Inc, Westbrook ME. Human TNFa and IL-1 were obtained from Collaborative Biomedical Products, Bedford MA. Dulbecco's modified Eagles medium (DMEM) and RPMI-1640 medium were purchased from JRH Biosciences, Lenexa KS. Fetal bovine serum (FBS) was purchased from Harlan, Indianapolis IN. Sterile Hank's balanced salt solution (HBSS) and phosphate buffered saline (PBS) were purchased from Bio Whittaker, Walkersville MD. Calcein AM was obtained from Molecular Probes, Eugene OR. Neuraminidase was purchased from Boehringer Mannheim, Indianapolis IN. All other reagents were of analytical grade or better and purchased from Sigma Chemical Co., Saint Louis MO, unless otherwise specified.

Cell culture: Ramos, Jurkat, and U-937 cells were obtained from the American Type Culture Collection. Ramos and Jurkat were maintained in RPMI 1640 supplemented with 10% heat-inactivated

FCS and 2 mM glutamine. U937 cells were maintained in RPMI 1640 supplemented with 15% FCS.

Porcine aortic endothelial cells (PAEC) were obtained at passage 1 (Cell Systems, Kirkland WA) and maintained in DMEM containing 10% FBS, penicillin 100 U/ml, and streptomycin 100 µg/ml (pen/strep, JRH Biosciences, Lenexa KS), hereinafter referred to as D10 medium. PAEC were at passage 2-4 in all assays. For cell binding assays, PAEC were removed from culture flasks with trypsin EDTA and replated onto 96 well culture dishes at a density of 1 x 10^4 cells/well. The human promyelocytic leukemia cell line HL-60 was obtained from the American Type Culture Collection (ATCC), Rockville, MD and maintained in D10.

Assays for Neutrophil / HL-60 binding to PAEC: Confluent monolayers of PAEC in 96 well plates were incubated (4 hr, 37°C) in 200 µl/well DMEM alone, DMEM containing 25 ng/ml human TNFa, or DMEM containing 10 ng/ml human IL-1. During this incubation, human neutrophils were isolated from 60 ml of human blood obtained from a healthy donor using the manufacturer's protocol (Polymorphoprep, Oslo, Norway), or HL-60 cells were spun down from culture medium.

The isolated neutrophils or HL-60 cells were washed 2x with HBSS, resuspended in HBSS containing 1% BSA (HBSS/BSA) at a final concentration of 3 x 10^6 cells/ml, incubated (30 min, 37°C) in the cytoplasmic indicator dye calcein AM (10 µM), washed 2x with HBSS and resuspended to 3 x 10^6 cells/ml in HBSS/BSA. Prior to addition to PAEC monolayers, the purified human neutrophils or HL-60 cells were incubated (30 min, 37°C) in either, HBSS/BSA, HBSS/BSA containing 0.25 U/ml neuraminidase, or HBSS/BSA containing 10 µg/ml anti-LFA-1 mAb.

Following this incubation, the neutrophils or HL-60 cells were washed 2x with HBSS/BSA and resuspended to 3 x 10^6 cells/ml. PAEC monolayers were then washed 3x with HBSS/BSA and calcein-loaded human neutrophils or HL-60 cells were added at 3 x 10^5 cells/well. The plates were centrifuged briefly (250 x g, 1 minute), incubated in the dark for 5 min at 37°C and then centrifuged upside down at 250 x g for 3 minutes. The media and unbound neutrophils or HL-60 cells were removed from the plate

and the bound cells were lysed by the addition of 1% SDS (100 µl/well) in HBSS. Neutrophil or HL-60 cell binding was determined by measuring the release of calcein from bound neutrophils or HL-60 cells into the lysis buffer using a Cytofluor 2350 (Millipore, Bedford MA -- excitation wavelength=485nm, emission wavelength=530nm). Background fluorescence was determined from wells containing PAEC that did not receive labeled neutrophils or HL-60 cells.

ELISA screen for anti-porcine cell interaction protein antibodies: To test antibodies for reactivity with porcine cell interaction proteins, an ELISA is carried out using the following protocol:

A 50 µL aliquot of a solution of a solublized (or soluble form of) a porcine cell interaction molecule is suspended in sodium carbonate/bicarbonate buffer, pH 9.5 and incubated overnight at 4°C in each test well of a 96 well plate (Nunc-Immuno F96 Polysorp, A/S Nunc, Roskilde, Denmark) in order to bind the protein to the plastic plate. The wells are then subjected to a wash step. (Each wash step consisted of three washes with TBST.) Next, test wells were blocked with 200 µL of blocking solution, 1% BSA in TBS (BSA/TBS), for 1 hour at 37°C (or, in some cases, 4°C overnight). After an additional wash step, a 50 µL aliquot of test antibody solution (e.g., hybridoma supernatant) is incubated in each test well for 1 hour at 37°C with a subsequent wash step.

As a secondary (detection) antibody, 50 µL of a 1:2000 dilution of horseradish peroxidase (HRP) conjugated goat anti-mouse IgG in BSA/TBS is incubated in each test well for 1 hour at 37°C, followed by a wash step. Following the manufacturer's procedures, 10 mg of O-phenylenediamine (Sigma Chemical Company, St. Louis, MO, Catalog No. P-8287) is dissolved in 25 mLs of phosphate-citrate buffer (Sigma Chemical Company, St. Louis, MO, Catalog No. P-4922), and 50 µL of this substrate solution is added to each well to allow detection of peroxidase activity. Finally, to stop the peroxidase detection reaction, a 50 µL aliquot of 3N hydrochloric acid is added to each well. The presence of antibodies reactive with a porcine cell interaction protein in the test antibody solutions is read out by a spectrophotometric OD determination at 490 nm.

The solution of porcine cell adhesion protein in sodium carbonate/bicarbonate buffer that serves as a source of the protein bound to the plastic plate is used at 2-fold serial dilutions across the plate starting at 50 µg of protein per mL, i.e., at 50, 25, 12.5, 6.25, 3.125, 1.5625, and 0.78125 µg/mL. These dilutions are used to determine the minimum amount of porcine cell interaction protein that will give maximum sensitivity in this assay.

Cloning of Porcine VCAM. Total RNA was prepared from TNFa-stimulated PAEC (25) and used to generate a porcine cDNA probe by reverse transcriptase-PCR using the following primers: 5' AAAAAAGCGGAGACAGGAGACA 3' and 5' TTCTGTGCTTCTACAAGACT 3'. The primer selection was based on sequence similarity between human, murine and rat VCAM. The resulting 299 bp PCR product was subcloned by TA-cloning into plasmid pCRII creating plasmid pCRIIpVCAM48 (Invitrogen, San Diego, CA). Plasmid pCRIIpVCAM48 was random primed and used to screen a TNFa-stimulated PAEC cDNA Uni-ZAP XR 1 library (25). A full-length, five Ig domain pVCAM cDNA was identified and entirely sequenced on both strands using a series of internal primers. The sequence for our porcine VCAM was identical to that reported by Tsang et al. (26) except for seven nucleotide differences between the two sequences at positions 185 (T>G), 655 (C>T), 815 (A>G), 1060 (C>T), 1120 (G>A), 1234 (A>C) and 1311 (C>T) which result in 4 amino acid changes at residues 30 (F>V), 240 (M>V), 379 (E>D), and 405 (T>I). This sequence has been submitted to the GenBank database under accession number L43124.

Cells. COS-7 and Ramos cells were obtained from the American Type Culture Collection. Ramos cells were maintained in RPMI 1640 supplemented with 10% heat-inactivated FBS and 2 mM glutamine. COS-7-7 and human 293-EBNA cells were grown as previously described (27). PAEC AND HUVECs were obtained (Cell Systems, Kirkland WA) at passage 1 and maintained as described (6) and used for adhesion assays or RNA isolation at passages 2-4. Human resting T cells were purified as previously described (6).

Construction of pVCAM and spVCAM Expression Vectors. The complete pVCAM coding region was cloned into the mammalian

expression vectors pAPEX-1 and pAPEX-3 (27). Plasmid pAPEX-1-pVCAM was transfected into COS-7 cells as described previously (27). A truncated version of pVCAM was constructed by deleting the transmembrane and cytoplasmic domains as follows. Briefly, the mammalian expression vector pAPEX-3/pVCAM was cleaved with *Nhe*I and *Sph*I and ligated to a 181 bp PCR fragment which supplied a six histidine tag and a stop codon using the following primers: 5'-CCCGAATTCGCATATACCATCCACAGG-3' and 5'-CGCGGA TCCTGCATGCATTAATGGTGATGGTGATGGTGTTCAGAAGAAAAATAGTCC-3'. This plasmid, pAPEX-3/spVCAM, encodes the signal sequence and extracellular domains of pVCAM.

Cell Adhesion Assays. Confluent monolayers of PAEC were used untreated or stimulated with 25 ng/ml of TNFa for 16-24 h. Ramos cells or human peripheral blood T cells were washed twice with RPMI containing 1.0% FBS (RPMI/1) and labeled with 10 mM Calcein-AM (Molecular Probes, Eugene OR) as previously described (6). Labeled cells (3 x 10^5 cells/ml) were washed twice with RPMI/1 and added (100 ml/well) to cell monolayers. The plates were gently centrifuged (50 x g, 1 min) and incubated in the dark for 30 min at 37°C in 5% CO_2. The plates were inverted and centrifuged at 250 x g for 3 min. Nonadherent cells were removed from the plate by gently washing five times with RPMI/1 and after a brief centrifugation, adherent cells were lysed by addition of 0.1 ml 1 % SDS to each well. Adherence was quantified by measuring the release of the fluorescent dye from bound cells using a Cytofluor 2350 fluorescent plate reader (Millipore, Bedford, MA) using an excitation wavelength of 485 nm and an emission wavelength of 530 nm. Background fluorescence was determined from wells containing COS-7 cells or PAEC that did not receive labeled cells. Test and control samples were performed in triplicate in each experiment. For the inhibition studies, labeled Ramos or human peripheral T cells were preincubated for 15 min at 37°C with mAb HP2/1 (anti-VLA-4) at 10 ug/ml prior to the adhesion assay. Blocking by anti-pVCAM mAbs was assessed in the continuous presence of the indicated concentrations of mAb.

Purification of spVCAM. The APEX-3/spVCAM expression vector was transfected into human 293-EBNA embryonic kidney cells (Invitrogen, San Diego, CA) as previously described (27). spVCAM was purified from concentrated serum-free conditioned medium from

293-EBNA cells expressing spVCAM by metal affinity chromatography using a nickel charged nitrilotriacetic acid (NTA) resin (Qiagen, Chatsworth, CA). Briefly, 200 ml of concentrated medium was adjusted to 20 mM Tris-Cl, pH 7.9, 500 mM NaCl, 5mM imidazole and incubated overnight with 5 ml Ni^{++}-NTA resin at 4°C with gentle agitation. The resin was washed with an additional 30 ml of binding buffer followed by 40 ml of wash buffer (20 mM Tris-Cl, pH 7.9, 500 mM NaCl, 60 mM imidazole). Finally, spVCAM was eluted with 9 ml elution buffer (20 mM Tris-Cl, pH 7.9, 500 mM NaCl, 1 M imidazole), concentrated with a Centriprep-30 (Amicon, Beverly MA), dialyzed extensively against PBS, sterile filtered and stored at 4°C. Protein concentration was determined by the Lowry method. Affinity purified spVCAM was subjected to SDS-PAGE, transferred to polyvinylidene difluoride membranes (Problot, Applied Biosystems) and sequenced directly using an Applied Biosystems 470A gas phase protein sequencer.

spVCAM Adhesion Assay. The ability of the truncated soluble form of pVCAM to support adhesion was assessed after immobilization on plastic. Briefly, recombinant spVCAM or BSA was precoated onto separate microtest wells (NUNC-Immuno Plate, Maxisorp) at the indicated concentrations in 100 ml binding buffer (15 mM sodium bicarbonate/35 mM sodium carbonate, pH 9.2) at 4°C overnight. The wells were blocked with RPMI 1640 containing 10 mg/ml BSA for 1 h at ambient temperature and washed once with RPMI containing 10% fetal bovine serum. Labeled Ramos cells (3 x 10^5 cells/well) were added to the wells, the plates centrifuged (50 x g, 1 min) and incubated at 37°C for 30 min. The nonadherent cells were removed by centrifugation of the sealed microtiter plate in the inverted position at 200 x *g* for 3 min and the bound cells lysed with 1.0 % SDS. The amount of released fluorochrome from lysed cells was determined as described above. For spVCAM binding inhibition studies, Ramos cells were preincubated with anti-human VLA-4 mAb (HP2/1) at 10 mg/ml for 15 min at 37°C or spVCAM coated wells were treated with varying concentrations of anti-pVCAM mAbs for 1 h at 37°C prior to the adhesion assays.

Antibodies. Blocking anti-a4-integrin (CD49d) mAb HP2/1 was purchased from Amac, Inc. (Westbrook, ME). Mouse anti-porcine

VCAM (anti-pVCAM) mAbs were prepared by intraperitoneal immunization of Balb/c mice with 100 mg of recombinant spVCAM in complete Freund's adjuvant. Following two boost injections with 100 mg of spVCAM in incomplete Freund's adjuvant, SP2/0 myeloma cells were fused using polyethylene glycol with spleen cells from the immunized animals. Hybridoma supernatants were screened 10-14 days later by ELISA for binding to spVCAM. Blocking anti-pVCAM mAbs were screened in a 30 min adhesion assay for the ability to inhibit the binding of Ramos cells to immobilized spVCAM and in a second adhesion assay for the ability to inhibit the binding of labeled Ramos cells to TNFa-stimulated PAEC (see below). Three anti-pVCAM mAbs (2A2, 3F4, 5D11) were selected for characterization. The mAbs were purified from ascites fluid on protein G-SEPHAROSE columns (Pharmacia, Piscataway, NJ) and are of the IgG1 isotype.

FACS analysis. Activated PAEC and HUVECs were analyzed for cell surface expression of VCAM using mouse anti-pVCAM mAb 2A2, 3F4, 5D11, or a commercially available mouse anti-hVCAM mAb (51-10C9; Pharmingen, San Diego, CA). Cells were treated with human TNFa (25 ng/ml) for approximately 24 h, harvested from culture flasks using mild trypsination and washed twice with PBS containing 2% FBS (PBS/2). Five hundred thousand cells were incubated with 5.0 mg/ml 3F4, 2A2, 5D11 or 51-10C9 for 1 h on ice. The cells were washed twice with PBS/2 and incubated for 30 min on ice with FITC-conjugated goat anti-mouse IgG (Zymed Laboratories, San Francisco, CA). The cells were washed in PBS/2 and analyzed by FACS using a Becton Dickerson FACSort (Becton Dickenson Immunocytometry Systems, San Jose, CA).

Epitope mapping by pairwise interaction analysis. F(ab')2 fragments were prepared by digestion of purified 2A2 and 3F4 mAb with ficin in the presence of 1 mM cysteine as described by the manufacturer (Pierce, Rockford, IL). Undigested mAb and Fc fragments were removed by subsequent protein A-sepharose chromatography. PolySorp microtiter plates (Nunc, Naperville, IL) were coated overnight at 4°C with 50 ml/well of 2 mg/ml 2A2 or 3F4 F(ab')2 in 0.1 M Na_2CO_3 pH 9.6. The plates were then washed three times with PBS containing 0.5% (v/v) Tween 20 and blocked with blocking buffer (PBS supplemented with 1% (w/v) BSA and 0.5% Tween 20) at 37°C for 1 h. The plates were washed and incubated

with 50 ml/well blocking buffer containing 2 mg/ml spVCAM at 37°C for 1 h. After additional washing, the plates were incubated at 37°C for 1 h with 50 ml/well blocking buffer or blocking buffer containing 1 mg/ml 2A2, 3F4, or 5D11 mAb. After washing the plates were incubated with 50 ml/well blocking buffer containing peroxidase-conjugated goat anti-mouse IgG Fc (Sigma, St. Louis, MO) at a 1:2000 dilution. After three final washes, the plate was developed with 50 ml/well substrate buffer (0.05 M phosphate-citrate buffer, pH 5.0/0.3 mg/ml sodium perborate/0.4 mg/ml o-phenylenediamine dihydrochloride). Reactions were stopped by the addition of 50 ml/well 1 M sulfuric acid. Quantitation was performed using a Bio-Rad model 3550 plate reader set at 490 nm.

Statistical analysis. Differences between the results of experimental treatments were evaluated by means of the Student's *t*-test.

RESULTS

COS-7 Cells Transiently Transfected with pVCAM cDNA Bind Human Ramos Cells in a VLA-4 Dependent Manner. To test the ability of pVCAM to support adhesion, we assayed the binding of Ramos cells to TNFa-stimulated PAEC and pVCAM-transfected COS cells. Labeled Ramos cells bound to TNFa-induced PAEC and pVCAM-transfected COS-7 cells (Fig. 1). In contrast, Ramos cells did not adhere to mock-transfected COS-7 cells.

To evaluate the predicted role of human a4b1 integrins (VLA-4) in pVCAM dependent cell-cell adhesion, an anti-human VLA-4 mAb was tested for its ability to inhibit adhesion of Ramos cells to pVCAM-transfected COS-7 cells and PAEC stimulated with TNFa. As shown in Fig. 1, the anti-VLA-4 mAb HP2/1 completely blocked the attachment of Ramos to both TNFa-activated PAEC and pVCAM-transfected COS-7 cells. Cell-cell adhesion was not blocked by an isotype-matched control antibody. Thus, pVCAM is a functional adhesion molecule and supports binding of TNFa-stimulated PAEC and pVCAM-transfected COS-7 cells to human lymphoid cells in a VLA-4-dependent manner.

VLA-4$^+$ Ramos Cells Specifically Adhere to Immobilized spVCAM. The spVCAM-(His)$_6$ used in this study was created by fusing a cDNA fragment encoding the extracellular domain of pVCAM (residues 1-497) to a sequence encoding a C terminal hexahistidine tag and a

stop codon at the leucine which is the first amino acid of the putative transmembrane domain (Fig. 2A). The resulting spVCAM was secreted into the culture medium of stably transfected 293-EBNA cells and purified by metal affinity chromatography to >90 % purity (Fig. 2B). spVCAM was subjected to 6 cycles of N-terminal sequencing. The sequence (VSQNVK) included four additional amino acids from that determined for the amino terminus of human VCAM (28), the putative termini for rat and mouse VCAM (29) and the pVCAM sequence recently reported by Tsang et al. (26). The secretion of spVCAM as a soluble protein, and its N-terminal sequence, confirms the assignment of the pVCAM signal sequence, transmembrane and cytoplasmic regions.

We examined the concentration dependence of the immobilized pVCAM on binding to labeled Ramos cells (Fig. 3A). A fixed number of labeled cells were incubated in wells precoated with the indicated concentrations of spVCAM. Ramos cells bound to immobilized spVCAM in a dose-dependent manner with saturation obtained at approximately 0.1 mg/well (Fig. 3A). Pretreatment of labeled Ramos cells with anti-VLA-4 mAb HP2/1 caused complete inhibition of spVCAM mediated binding (Fig. 3B). In control experiments, Ramos cells failed to bind to immobilized BSA (Fig. 3B). These results demonstrate that soluble spVCAM mediates binding of human VLA-4^+ target cells.

Anti-pVCAM mAbs. Having established the interaction of human VLA-4 with pVCAM, we investigated the potential of inhibiting this interaction with blocking mAbs to pVCAM. Hybridomas were derived from the spleen cells of Balb/c mice immunized with spVCAM and used to make hybridomas. Numerous mAbs were produced that recognized pVCAM by ELISA and FACS analysis (data not shown). Several mAbs were tested in a rapid screening assay involving the adherence of Ramos cells to immobilized spVCAM. Two mAbs, 2A2 and 3F4, significantly inhibited Ramos cell binding in a dose-dependent manner (Fig. 4). Anti-pVCAM mAb 3F4 completely blocked Ramos cell binding to spVCAM at a concentration of 3 mg/ml, where as mAb 2A2 maximally inhibited binding to pVCAM at a higher concentration (30 mg/ml) (Fig. 4). The weaker inhibition observed with the anti-pVCAM mAb 2A2 may reflect its reactivity with a distinct epitope on the pVCAM molecule (see below). In

contrast, a third anti-pVCAM mAb, 5D11, showed virtually no inhibitory effect, even at high concentrations (Fig. 4).

To characterize the specificity of these mAbs to pVCAM, FACS analysis was performed on cytokine-stimulated HUVEC and PAEC. The anti-pVCAM mAbs 2A2 and 3F4 all reacted with TNFa-stimulated PAEC but did not react with TNFa-stimulated HUVEC cells (Fig. 5). Of the nonblocking mAbs, 5D11, was also shown to be specific for PAEC (Fig. 5). In contrast, the anti-human VCAM-1 mAb, 51-10C9, reacted with stimulated HUVEC but did not cross react with cell surface pVCAM present on stimulated PAEC, indicating that mAbs 2A2, 3F4 and 5D11 recognize porcine-specific epitopes. Flow cytometric analysis also revealed that pVCAM was highly expressed on LPS activated PAEC, whereas recombinant human IL-1 did not induce VCAM expression on PAEC (data not shown).

Epitope mapping of the anti-pVCAM mAbs was performed by pairwise interaction analysis. This approach tested the ability of mAb pairs to bind simultaneously to spVCAM. As shown in Fig. 6, mAbs 2A2 and 3F4 did not interfere with the binding of the remaining mAbs to spVCAM. Therefore, the mAb epitopes are nonoverlapping and represent distinct antigenic regions on the pVCAM molecule.

Inhibition of Ramos and Human T Cell Binding to Cytokine-Activated PAEC by Anti-pVCAM mAbs. We next tested the ability of mAbs 2A2 and 3F4 to block Ramos and human T cell binding to stimulated PAEC. The mAbs 3F4 and 2A2 inhibited Ramos cell binding to activated PAEC by >90% (Fig. 7). In analogous fashion, adhesion of human T cells to stimulated PAEC was blocked (~65%) by the same mAbs ($p<0.01$, Fig. 7). The anti-pVCAM mAbs 2A2 and 3F4 inhibited binding of human T cells to TNFa-stimulated PAEC to the same degree as the anti-VLA-4 mAb (Fig. 7). The degree of anti-pVCAM mAb-mediated inhibition of T cell interaction with PAEC was less than for Ramos binding to PAEC, suggesting that adhesion interactions other than VLA-4/VCAM are likely to play a role in human T cell/PAEC adhesion. Nevertheless, the data demonstrate a major role for pVCAM in mediating PAEC adhesion to human lymphocytes.

Recombinant Expression of anti-VCAM Antibodies

Standard molecular biology techniques were used (Sambrook et al., 1989). Cloning of the variable regions from the hybridomas 2A2

and 3F4 was performed using a set of commercially available primers (Mouse Ig-Primer Set, Novagen, Madison, WI) as described previously (Evans et al, In press). Chimeric antibodies were produced by cloning the 2A2 and 3F4 variable regions into the expression plasmid pAPEX-3P (Evans et al., 1995) modified to contain the human gamma4 constant region in place of the human gamma 1 C1 region. The resulting expression plasmids were transfected into 293-EBNA cells and selected for puromycin resistance as described previously (Evans et al., 1995). Upon reaching confluence, cells were refed serum-free HB PRO (Irvine Scientific, Santa Ana, CA) every 3 to 4 days. The conditioned medium was centrifuged at 4500 x g to remove cell debris, concentrated 10-fold, and dialyzed into 20 mM sodium phosphate, pH 7.0. Antibody was subsequently purified using a 1 ml HiTrap Protein A column (Pharmacia, Piscataway, NJ), dialyzed into PBS, passed through a 0.2 micron filter, and stored at 4°C. $F(ab')_2$ and Fab were produced by digestion of murine monoclonal antibody or chimeric antibody with Ficin (Pierce, Rockford, IL) or papain (Pierce), respectively, followed by protein-A chromatography to remove undigested antibody and Fc fragments.

The antibodies were tested for function as described above.

1. ANTIBODY SEQUENCES

2A2 LIGHT CHAIN DNA SEQUENCE

```
4461/1                                                  4491/11
ATG GGC TTC AAG ATG GAG TCA CAT TTT CAG GTC TTT GTA TAC ATG TTG CTG TGG TTG TCT
M   G   F   K   M   E   S   H   F   Q   V   F   V   Y   M   L   L   W   L   S
4521/21                                                 4551/31
GGT GTT AAT GGA GAC ATT GTG ATG ACC CAG TCT CAA AAA TTC ATG TCC ACA TCA TTA GGA
G   V   N   G   D   I   V   M   T   Q   S   Q   K   F   M   S   T   S   L   G
4581/41                                                 4611/51
GAC AGG GTC AGC GTC ACC TGC AAG GCC AGT CAG AAT GTG GGT CCT AAT GTA GCC TGG TTT
D   R   V   S   V   T   C   K   A   S   Q   N   V   G   P   N   V   A   W   F
4641/61                                                 4671/71
CAA CAG AAA CCA GGC CAG TCT CCT AAA ACA CTT ATT TAC TCG GCA TCC TTC CGC TAC AGT
Q   Q   K   P   G   Q   S   P   K   T   L   I   Y   S   A   S   F   R   Y   S
4701/81                                                 4731/91
GGA GTC CCT GAT CGC TTC ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC ACC
G   V   P   D   R   F   T   G   S   G   S   G   T   D   F   T   L   T   I   T
4761/101                                                4791/111
AAT GTG CAG TCT GAA GAC TTG GCA GAG TAT TTC TGT CAT CAA TAT AAC TCC TAT CCT CTC
N   V   Q   S   E   D   L   A   E   Y   F   C   H   Q   Y   N   S   Y   P   L
4821/121                                                4851/131
ACG TTC GGG GGG GGG ACC AAG CTG AAA ATA AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC
T   F   G   G   G   T   K   L   K   I   K   R   T   V   A   A   P   S   V   F
4881/141                                                4911/151
ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG
I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L
4941/161                                                4971/171
AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG
N   N   F   Y   P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S
5001/181                                                5031/191
GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC
G   N   S   Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S
5061/201                                                5091/211
AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC
S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V
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5121/221                                                    5151/231
ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG
T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E   C   *
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2A2 HEAVY CHAIN cDNA SEQUENCE

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1/1                                                         31/11
ATG GGA TGG AGC TAT ATC ATG TTC TTC TTG GTA TCA ACA GCT ACA GTT GTC CAC TCC CAG
M   G   W   S   Y   I   M   F   F   L   V   S   T   A   T   V   V   H   S   Q
61/21                                                       91/31
GTA CAA CTG CAG CAG TCT GGG CCT CAG CTG GTT AGG CCT GGG ACT TCA GTG AAG ATA TCC
V   Q   L   Q   Q   S   G   P   Q   L   V   R   P   G   T   S   V   K   I   S
121/41                                                      151/51
TGC AAG GCT TCT GGT TAC TCA TTC ACC AGC TAT TGG ATG CAC TGG GTG AAG CAG AGG CCT
C   K   A   S   G   Y   S   F   T   S   Y   W   M   H   W   V   K   Q   R   P
181/61                                                      211/71
GGA CAA GAT CTT GAG TGG ATT GGC ATG ATT GAT CCA TCC GAT AGT GAA GTT AAA TTA AAT
G   Q   D   L   E   W   I   G   M   I   D   P   S   D   S   E   V   K   L   N
241/81                                                      271/91
CAG AGG TTA AAG GAC AAG GCC ATA TTG ACT GTT GAC AAA TCC TCC AAC ACA GCC TAC ATG
Q   R   L   K   D   K   A   I   L   T   V   D   K   S   S   N   T   A   Y   M
301/101                                                     331/111
CAA TTC AGC GGC CCG ACT TCT GAG GAC TCT GCG GTC TAT TAC TGT ACA AGA GGG GAG GTT
Q   F   S   G   P   T   S   E   D   S   A   V   Y   Y   C   T   R   G   E   V
361/121                                                     391/131
TCC TGG TTT GCT TAC TGG GGC CAG GGG ACT CTG GTC ACT GTC TCT GCA GCC TCC ACC AAG
S   W   F   A   Y   W   G   Q   G   T   L   V   T   V   S   A   A   S   T   K
421/141                                                     451/151
GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC GCC
G   P   S   V   F   P   L   A   P   C   S   R   S   T   S   E   S   T   A   A
481/161                                                     511/171
CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC
L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S   G
541/181                                                     571/191
GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC
A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L   Y   S
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601/201                                                 631/211
CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC
L   S   S   V   V   T   V   P   S   S   S   L   G   T   K   T   Y   T   C   N
661/221                                                 691/231
GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GAG TCC AAA TAT GGT CCC
V   D   H   K   P   S   N   T   K   V   D   K   R   V   E   S   K   Y   G   P
721/241                                                 751/251
CCA TGC CCA TCA TGC CCA GCA CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC
P   C   P   S   C   P   A   P   E   F   L   G   G   P   S   V   F   L   F   P
781/261                                                 811/271
CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG
P   K   P   K   D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V
841/281                                                 871/291
GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG
D   V   S   Q   E   D   P   E   V   Q   F   N   W   Y   V   D   G   V   E   V
901/301                                                 931/311
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC
H   N   A   K   T   K   P   R   E   E   Q   F   N   S   T   Y   R   V   V   S
961/321                                                 991/331
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S
1021/341                                                1051/351
AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA
N   K   G   L   P   S   S   I   E   K   T   I   S   K   A   K   G   Q   P   R
1081/361                                                1111/371
GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC
E   P   Q   V   Y   T   L   P   P   S   Q   E   E   M   T   K   N   Q   V   S
1141/381                                                1171/391
CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT
L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N
1201/401                                                1231/411
GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC
G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F
1261/421                                                1291/431
TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA
F   L   Y   S   R   L   T   V   D   K   S   R   W   Q   E   G   N   V   F   S
1321/441                                                1351/451
TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT
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C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S
1381/461
CTG GGT AAA TGA
L   G   K   *
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2A2 (CHIMERIC) HUMAN G2/G4 cDNA

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1/1                                                 31/11
ATG GGA TGG AGC TAT ATC ATG TTC TTC TTG GTA TCA ACA GCT ACA GTT GTC CAC TCC CAG
M   G   W   S   Y   I   M   F   F   L   V   S   T   A   T   V   V   H   S   Q
61/21                                               91/31
GTA CAA CTG CAG CAG TCT GGG CCT CAG CTG GTT AGG CCT GGG ACT TCA GTG AAG ATA TCC
V   Q   L   Q   Q   S   G   P   Q   L   V   R   P   G   T   S   V   K   I   S
121/41                                              151/51
TGC AAG GCT TCT GGT TAC TCA TTC ACC AGC TAT TGG ATG CAC TGG GTG AAG CAG AGG CCT
C   K   A   S   G   Y   S   F   T   S   Y   W   M   H   W   V   K   Q   R   P
181/61                                              211/71
GGA CAA GAT CTT GAG TGG ATT GGC ATG ATT GAT CCA TCC GAT AGT GAA GTT AAA TTA AAT
G   Q   D   L   E   W   I   G   M   I   D   P   S   D   S   E   V   K   L   N
241/81                                              271/91
CAG AGG TTA AAG GAC AAG GCC ATA TTG ACT GTT GAC AAA TCC TCC AAC ACA GCC TAC ATG
Q   R   L   K   D   K   A   I   L   T   V   D   K   S   S   N   T   A   Y   M
301/101                                             331/111
CAA TTC AGC GGC CCG ACT TCT GAG GAC TCT GCG GTC TAT TAC TGT ACA AGA GGG GAG GTT
Q   F   S   G   P   T   S   E   D   S   A   V   Y   Y   C   T   R   G   E   V
361/121                                             391/131
TCC TGG TTT GCT TAC TGG GGC CAG GGG ACT CTG GTC ACT GTC TCT GCA GCC TCC ACC AAG
S   W   F   A   Y   W   G   Q   G   T   L   V   T   V   S   A   A   S   T   K
421/141                                             451/151
GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC GCC
G   P   S   V   F   P   L   A   P   C   S   R   S   T   S   E   S   T   A   A
481/161                                             511/171
CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC
L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S   G
541/181                                             571/191
GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC
A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L   Y   S
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601/201 631/211
CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AAC TTC GGC ACC CAG ACC TAC ACC TGC AAC
L S S V V T V P S S N F G T Q T Y T C N
661/221 691/231
GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG ACA GTT GAG CGC AAA TGT TGT GTC
V D H K P S N T K V D K T V E R K C C V
721/241 751/251
GAG TGC CCA CCG TGC CCA GCA CCA CCT GTG GCA GGA CCG TCA GTC TTC CTC TTC CCC CCA
E C P P C P A P P V A G P S V F L F P P
781/261 811/271
AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG GAC
K P K D T L M I S R T P E V T C V V V D
841/281 871/291
GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG CAT
V S Q E D P E V Q F N W Y V D G V E V H
901/301 931/311
AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC
N A K T K P R E E Q F N S T Y R V V S V
961/321 991/331
CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC
L T V L H Q D W L N G K E Y K C K V S N
1021/341 1051/351
AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA GAG
K G L P S S I E K T I S K A K G Q P R E
1081/361 1111/371
CCA CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG
P Q V Y T L P P S Q E E M T K N Q V S L
1141/381 1171/391
ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG
T C L V K G F Y P S D I A V E W E S N G
1201/401 1231/411
CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC
Q P E N N Y K T T P P V L D S D G S F F
1261/421 1291/431
CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA TGC
L Y S R L T V D K S R W Q E G N V F S C

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1321/441                                    1351/451
TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG
S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S   L
1381/461
GGT AAA TGA
G   K   *
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2A2 HUMAN G2/G4 EXPERSSION PLASMID INSERT SEQUENCE

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   1 gtgaccaatacaaaacaaaagcgcccctcgtaccagcgaagaaggggcagagatgccgtagtcaggtttagttcgtccgg  80
  81 cggcgggggatctgtatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatctgctccctgctt 160
 161 gtgtgttggaggtcgctgagtagtgcgcgagcaaaatttaagctacaacaaggcaaggcttgaccgacaattgcatgaag 240
 241 aatctgcttagggttaggcgttttgcgctgcttcgcgatgtacgggccagatatacgcgttgacattgattattgactag 320
 321 ttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatg 400
 401 gcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggg 480
 481 actttccattgacgtcaatgggtggactatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaag 560
 561 tacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttccta 640
 641 cttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatag 720
 721 cggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacggga 800
 801 ctttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagca 880
 881 gagctcgtttagtgaaccgtcaGAATTCTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTG 960
 961 GATCGGAAACCCGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAAC 1040
1041 CTCTCGACTGTTGGGgtgagtactccctctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgag 1120
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1121 gaggatttgatattcacctggcccgcggtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatcttttt 1200

1201 gttgtcaagcttgaggtgtggcaggcttgagatctggccatacacttgagtgacaatgacatccactttgcctttctctc 1280

1281 cacagGTGTCCACTCCCAGGTCCAACTGCAGGTCGAC ATG GGA TGG AGC TAT ATC ATG TTC TTC TTG    1347
   1                                      M   G   W   S   Y   I   M   F   F   L      10

1348 GTA TCA ACA GCT ACA GTT GTC CAC TCC CAG GTA CAA CTG CAG CAG TCT GGG CCT CAG CTG  1407
  11 V   S   T   A   T   V   V   H   S   Q   V   Q   L   Q   Q   S   G   P   Q   L    30

1408 GTT AGG CCT GGG ACT TCA GTG AAG ATA TCC TGC AAG GCT TCT GGT TAC TCA TTC ACC AGC  1467
  31 V   R   P   G   T   S   V   K   I   S   C   K   A   S   G   Y   S   F   T   S    50

1468 TAT TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GAT CTT GAG TGG ATT GGC ATG ATT  1527
  51 Y   W   M   H   W   V   K   Q   R   P   G   Q   D   L   E   W   I   G   M   I    70

1528 GAT CCA TCC GAT AGT GAA GTT AAA TTA AAT CAG AGG TTA AAG GAC AAG GCC ATA TTG ACT  1587
  71 D   P   S   D   S   E   V   K   L   N   Q   R   L   K   D   K   A   I   L   T    90

1588 GTT GAC AAA TCC TCC AAC ACA GCC TAC ATG CAA TTC AGC GGC CCG ACT TCT GAG GAC TCT  1647
  91 V   D   K   S   S   N   T   A   Y   M   Q   F   S   G   P   T   S   E   D   S   110

1648 GCG GTC TAT TAC TGT ACA AGA GGG GAG GTT TCC TGG TTT GCT TAC TGG GGC CAG GGG ACT  1707
 111 A   V   Y   Y   C   T   R   G   E   V   S   W   F   A   Y   W   G   Q   G   T   130

1708 CTG GTC ACT GTC TCT GCA GCC TCC ACC AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC  1767
 131 L   V   T   V   S   A   A   S   T   K   G   P   S   V   F   P   L   A   P   C   150

1768 TCC AGG AGC ACC TCC GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC  1827
 151 S   R   S   T   S   E   S   T   A   A   L   G   C   L   V   K   D   Y   F   P   170

1828 GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG  1887
 171 E   P   V   T   V   S   W   N   S   G   A   L   T   S   G   V   H   T   F   P   190

1888 GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC  1947
 191 A   V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T   V   P   S   S   210
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1948 AAC TTC GGC ACC CAG ACC TAC ACC TGC AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG  2007
 211 N   F   G   T   Q   T   Y   T   C   N   V   D   H   K   P   S   N   T   K   V    230

2008 GAC AAG ACA GTT G gtgagaggccagctcagggagggagggtgtctgctggaagccaggctcagccctcctgcctg 2082
 231 D   K   T   V   E                                                               235

2083 gacgcaccccggctgtgcagccccagcccagggcagcaaggcaggccccatctgtctcctcacccggaggcctctgcccg 2162

2163 ccccactcatgctcagggagagggtcttctggctttttccaccaggctccaggcaggcacaggctgggtgcccctacccc 2242

2243 aggcccttcacacacaggggcaggtgcttggctcagacctgccaaaagccatatccgggaggaccctgcccctgacctaa 2322

2323 gccgaccccaaaggccaaactgtccactccctcagctcggacaccttctctcctcccagatccgagtaactcccaatctt 2402

2403 ctctctgcag AG CGC AAA TGT TGT GTC GAG TGC CCA CCG TGC CCA G gtaagccagcccaggcctcg 2468
 236              R   K   C   C   V   E   C   P   P   C   P   A                    247

2469 ccctccagctcaaggcgggacaggtgccctagagtagcctgcatccagggacaggccccagctgggtgctgacacgtcca 2548

2549 cctccatctcttcctcag CA CCA CCT GTG GCA GGA CCG TCA GTC TTC CTC TTC CCC CCA AAA     2610
 248                     P   P   V   A   G   P   S   V   F   L   F   P   P   K       261

2611 CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG GAC GTG  2670
 262 P   K   D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V    281

2671 AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG CAT AAT  2730
 282 S   Q   E   D   P   E   V   Q   F   N   W   Y   V   D   G   V   E   V   H   N    301

2731 GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC  2790
 302 A   K   T   K   P   R   E   E   Q   F   N   S   T   Y   R   V   V   S   V   L    321

2791 ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA  2850
 322 T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K    341

2851 GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA G gtgggacccacggggtgcgagg 2915
 342 G   L   P   S   S   I   E   K   T   I   S   K   A   K   G                    356

2916 gccacacggacagaggccagctcggcccaccctctgccctgggagtgaccgctgtgccaacctctgtccctacag GG  2992
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2993 CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC  3052
 357 Q   P   R   E   P   Q   V   Y   T   L   P   P   S   Q   E   E   M   T   K   N    376

3053 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG  3112
 377 Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W    396

3113 GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC  3172
 397 E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D   S   D    416

3173 GGC TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT  3232
 417 G   S   F   F   L   Y   S   R   L   T   V   D   K   S   R   W   Q   E   G   N    436

3233 GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC  3292
 437 V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S   L    456

3293 TCC CTG TCT CTG GGT AAA TGA gtgccagggccattgaagcatttatcagggttattgtctcatgagcggatac 3365
 457 S   L   S   L   G   K   *                                                        463

3366 atatttgaatgtatttagaaaaataaacaaatagg                                             3400
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2A2 HUMAN IgG 4 EXPRESSION PLASMID INSERT SEQUENCE

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   1 gtgaccaatacaaaacaaaagcgcccctcgtaccagcgaagaaggggcagagatgccgtagtcaggtttagttcgtccgg  80

  81 cggcgggggatctgtatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatctgctccctgctt 160

 161 gtgtgttggaggtcgctgagtagtgcgcgagcaaaatttaagctacaacaaggcaaggcttgaccgacaattgcatgaag 240

 241 aatctgcttagggttaggcgttttgcgctgcttcgcgatgtacgggccagatatacgcgttgacattgattattgactag 320

 321 ttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatg 400

 401 gcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaataggg 480

 481 actttccattgacgtcaatgggtggactatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaag 560
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 561 tacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctа 640

 641 cttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatag 720

 721 cggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacggga 800

 801 ctttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagca 880

 881 gagctcgtttagtgaaccgtcaGAATTCTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTG 960

 961 GATCGGAAACCCGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAAC 1040

1041 CTCTCGACTGTTGGGgtgagtactccctctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgag 1120

1121 gaggatttgatattcacctggcccgcggtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatcttttt 1200

1201 gttgtcaagcttgaggtgtggcaggcttgagatctggccatacacttgagtgacaatgacatccactttgcctttctctc 1280

1281 cacagGTGTCCACTCCCAGGTCCAACTGCAGGTCGAC ATG GGA TGG AGC TAT ATC ATG TTC TTC TTG    1347
   1                                      M   G   W   S   Y   I   M   F   F   L      10

1348 GTA TCA ACA GCT ACA GTT GTC CAC TCC CAG GTA CAA CTG CAG CAG TCT GGG CCT CAG CTG  1407
  11 V   S   T   A   T   V   V   H   S   Q   V   Q   L   Q   Q   S   G   P   Q   L    30

1408 GTT AGG CCT GGG ACT TCA GTG AAG ATA TCC TGC AAG GCT TCT GGT TAC TCA TTC ACC AGC  1467
  31 V   R   P   G   T   S   V   K   I   S   C   K   A   S   G   Y   S   F   T   S    50

1468 TAT TGG ATG CAC TGG GTG AAG CAG AGG CCT GGA CAA GAT CTT GAG TGG ATT GGC ATG ATT  1527
  51 Y   W   M   H   W   V   K   Q   R   P   G   Q   D   L   E   W   I   G   M   I    70

1528 GAT CCA TCC GAT AGT GAA GTT AAA TTA AAT CAG AGG TTA AAG GAC AAG GCC ATA TTG ACT  1587
  71 D   P   S   D   S   E   V   K   L   N   Q   R   L   K   D   K   A   I   L   T    90

1588 GTT GAC AAA TCC TCC AAC ACA GCC TAC ATG CAA TTC AGC GGC CCG ACT TCT GAG GAC TCT  1647
  91 V   D   K   S   S   N   T   A   Y   M   Q   F   S   G   P   T   S   E   D   S   110
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1648 GCG GTC TAT TAC TGT ACA AGA GGG GAG GTT TCC TGG TTT GCT TAC TGG GGC CAG GGG ACT 1707
 111 A   V   Y   Y   C   T   R   G   E   V   S   W   F   A   Y   W   G   Q   G   T   130

1708 CTG GTC ACT GTC TCT GCA GCC TCC ACC AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC 1767
 131 L   V   T   V   S   A   A   S   T   K   G   P   S   V   F   P   L   A   P   C   150

1768 TCC AGG AGC ACC TCC GAG AGC ACA GCC GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC 1827
 151 S   R   S   T   S   E   S   T   A   A   L   G   C   L   V   K   D   Y   F   P   170

1828 GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG 1887
 171 E   P   V   T   V   S   W   N   S   G   A   L   T   S   G   V   H   T   F   P   190

1888 GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC 1947
 191 A   V   L   Q   S   S   G   L   Y   S   L   S   S   V   V   T   V   P   S   S   210

1948 AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG 2007
 211 S   L   G   T   K   T   Y   T   C   N   V   D   H   K   P   S   N   T   K   V   230

2008 GAC AAG AGA GTT G gtgagaggccagcacagggagggagggtgtctgctggaagccaggctcagccctcctgcctg 2082
 231 D   K   R   V   E                                                               235

2083 gacgcaccccggctgtgcagccccagcccagggcagcaaggcatgccccatctgtctcctcacccggaggcctctgacca 2162

2163 ccccactcatgctcagggagagggtcttctggatttttccaccaggctcccggcaccacaggctggatgcccctacccca 2242

2243 ggccctgcgcatacagggcaggtgctgcgctcagacctgccaagagccatatccgggaggaccctgcccctgacctaagc 2322

2323 ccaccccaaaggccaaactctccactccctcagctcagacaccttctctcctcccagatctgagtaactcccaatcttct 2402

2403 ctctgcag AG TCC AAA TAT GGT CCC CCA TGC CCA TCA TGC CCA G gtaagccaacccaggcctcgcc 2468
 236          S   K   Y   G   P   P   C   P   S   C   P   A                       247

2469 ctccagctcaaggcgggacaggtgccctagagtagcctgcatccagggacaggccccagccgggtgctgacgcatccacc 2548

2549 tccatctcttcctcag CA CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC CCC CCA AAA 2611
 248                 P   E   F   L   G   G   P   S   V   F   L   F   P   P   K   262
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2612 CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG GAC GTG  2671
 263 P   K   D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V   D   V    282

2672 AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG CAT AAT  2731
 283 S   Q   E   D   P   E   V   Q   F   N   W   Y   V   D   G   V   E   V   H   N    302

2732 GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC GTC CTC  2791
 303 A   K   T   K   P   R   E   E   Q   F   N   S   T   Y   R   V   V   S   V   L    322

2792 ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC AAC AAA  2851
 323 T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S   N   K    342

2852 GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA G gtgggacccacggggtgcgagg  2916
 343 G   L   P   S   S   I   E   K   T   I   S   K   A   K   G                        357

2917 gccacacggacagaggccagctcggcccaccctctgccctgggagtgaccgctgtgccaacctctgtccctacag GG   2993

2994 CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC  3053
 358 Q   P   R   E   P   Q   V   Y   T   L   P   P   S   Q   E   E   M   T   K   N    377

3054 CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG  3113
 378 Q   V   S   L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W    397

3114 GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC  3173
 398 E   S   N   G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D   S   D    417

3174 GGC TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT  3233
 418 G   S   F   F   L   Y   S   R   L   T   V   D   K   S   R   W   Q   E   G   N    437

3234 GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC  3293
 438 V   F   S   C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S   L    457

3294 TCC CTG TCT CTG GGT AAA TGA gtgccagggccattgaagcatttatcagggttattgtctcatgagcggatac  3366
 458 S   L   S   L   G   K   *                                                        464

3367 atatttgaatgtatttagaaaaataaacaaatagggttccgcgcacatttccccgaaaagtgccacctgacgcgttgac  3446

3447 attgattattgactagttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttaca  3526
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3527 taacttacggtaaatggccccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttccca 3606

3607 tagtaacgccaatagggactttccattgacgtcaatgggtggactatttacggtaaactgcccacttggcagtacatcaa 3686

3687 gtgtatcatatgccaagtacgcccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgac 3766

3767 cttatgggactttcctacttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtaca 3846

3847 tcaatgggcgtggatagcggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttgg 3926

3927 caccaaaatcaacgggactttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtg 4006

4007 ggaggtctatataagcagagctcgtttagtgaaccgtcaGAATTCTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGG 4086

4087 TCTTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCAT 4166

4167 CGACCGGATCGGAAAACCTCTCGACTGTTGGGgtgagtactccctctcaaaagcgggcatgacttctgcgctaagattgt 4246

4247 cagtttccaaaaacgaggaggatttgatattcacctggcccgcggtgatgcctttgagggtggccgcgtccatctggtca 4326

4327 gaaaagacaatctttttgttgtcaagcttgaggtgtggcaggcttgagatctggccatacacttgagtgacaatgacatc 4406

4407 cactttgcctttctctccacagGTGTCCACTCCCAGGTCCAACTGCAGGTCGAC ATG GGC TTC AAG ATG GAG   4478
   1                                                        M   G   F   K   M   E     6

4479 TCA CAT TTT CAG GTC TTT GTA TAC ATG TTG CTG TGG TTG TCT GGT GTT AAT GGA GAC ATT   4538
   7 S   H   F   Q   V   F   V   Y   M   L   L   W   L   S   G   V   N   G   D   I    26

4539 GTG ATG ACC CAG TCT CAA AAA TTC ATG TCC ACA TCA TTA GGA GAC AGG GTC AGC GTC ACC   4598
  27 V   M   T   Q   S   Q   K   F   M   S   T   S   L   G   D   R   V   S   V   T    46

4599 TGC AAG GCC AGT CAG AAT GTG GGT CCT AAT GTA GCC TGG TTT CAA CAG AAA CCA GGC CAG   4658
  47 C   K   A   S   Q   N   V   G   P   N   V   A   W   F   Q   Q   K   P   G   Q    66

4659 TCT CCT AAA ACA CTT ATT TAC TCG GCA TCC TTC CGC TAC AGT GGA GTC CCT GAT CGC TTC   4718
  67 S   P   K   T   L   I   Y   S   A   S   F   R   Y   S   G   V   P   D   R   F    86
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4719 ACA GGC AGT GGA TCT GGG ACA GAT TTC ACT CTC ACC ATC ACC AAT GTG CAG TCT GAA GAC  4778
  87 T   G   S   G   S   G   T   D   F   T   L   T   I   T   N   V   Q   S   E   D    106

4779 TTG GCA GAG TAT TTC TGT CAT CAA TAT AAC TCC TAT CCT CTC ACG TTC GGG GGG GGG ACC  4838
 107 L   A   E   Y   F   C   H   Q   Y   N   S   Y   P   L   T   F   G   G   G   T    126

4839 AAG CTG AAA ATA AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC ATC TTC CCG CCA TCT GAT  4898
 127 K   L   K   I   K   R   T   V   A   A   P   S   V   F   I   F   P   P   S   D    146

4899 GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG AAT AAC TTC TAT CCC AGA  4958
 147 E   Q   L   K   S   G   T   A   S   V   V   C   L   L   N   N   F   Y   P   R    166

4959 GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG GGT AAC TCC CAG GAG AGT  5018
 167 E   A   K   V   Q   W   K   V   D   N   A   L   Q   S   G   N   S   Q   E   S    186

5019 GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC AGC ACC CTG ACG CTG AGC  5078
 187 V   T   E   Q   D   S   K   D   S   T   Y   S   L   S   S   T   L   T   L   S    206

5079 AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC ACC CAT CAG GGC CTG AGC  5138
 207 K   A   D   Y   E   K   H   K   V   Y   A   C   E   V   T   H   Q   G   L   S    226

5139 TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG ctcgagcatgcaggcatgcaagcttggc 5205
 227 S   P   V   T   K   S   F   N   R   G   E   C   *                               239

5206 actggccgtcgttttacaacgtcgtgactgggaaaaccctggcgt                                   5250
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3F4 LIGHT CHAIN cDNA

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4496/1          c
ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GTT TCC AGC AGT GAT
M   K   L   P   V   R   L   L   V   L   M   F   W   I   P   V   S   S   S   D
4556/21                                 4586/31
GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA GCC TCC ATC
V   V   M   T   Q   T   P   L   S   L   P   V   S   L   G   D   Q   A   S   I
```

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4616/41                                                 4646/51
TCT TGC AGA TCT AGT CAG AGC CTT GTA CAC AGT AAT GGA AAC ACC TAT TTA CAG TGG TAC
S   C   R   S   S   Q   S   L   V   H   S   N   G   N   T   Y   L   Q   W   Y
4676/61                                                 4706/71
CTG CAG AAG CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC CGA TTT TCT
L   Q   K   P   G   Q   S   P   K   L   L   I   Y   K   V   S   N   R   F   S
4736/81                                                 4766/91
GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC AAG ATC AGC
G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T   L   K   I   S
4796/101                                                4826/111
AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TTC TGT TCT CAA AGT ACA CAT GTT CCG TTC
R   V   E   A   E   D   L   G   V   Y   F   C   S   Q   S   T   H   V   P   F
4856/121                                                4886/131
ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGA ACT GTG GCT GCA CCA TCT GTC TTC
T   F   G   G   G   T   K   L   E   I   K   R   T   V   A   A   P   S   V   F
4916/141                                                4946/151
ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG TGC CTG CTG
I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V   C   L   L
4976/161                                                5006/171
AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC CTC CAA TCG
N   N   F   Y   P   R   E   A   K   V   Q   W   K   V   D   N   A   L   Q   S
5036/181                                                5066/191
GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC AGC CTC AGC
G   N   S   Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y   S   L   S
5096/201                                                5126/211
AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC TGC GAA GTC
S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A   C   E   V
5156/221                                                5186/231
ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG TGT TAG
T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E   C   *
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3F4 HEAVY CHAIN cDNA

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1/1                                                     31/11
ATG AAG TGG AGC TGG GTT ATT CTC TTC CTC CTG TCA GTA ACT GCC GGC GTC CAC TCC CAG
M   K   W   S   W   V   I   L   F   L   L   S   V   T   A   G   V   H   S   Q
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61/21                                                   91/31
GTT CAG GTC CAG CAG TCT GGG GCT GAG CTG GCA AGA CCT TGG GCT TCA GTG AAG TTG TCC
V   Q   V   Q   Q   S   G   A   E   L   A   R   P   W   A   S   V   K   L   S
121/41                                                  151/51
TGC AAG GCT TCT GGC TAC AAT TTT AAT AGT TAC TGG ATG CAG TGG GTA AAA CAG AGG CCT
C   K   A   S   G   Y   N   F   N   S   Y   W   M   Q   W   V   K   Q   R   P
181/61                                                  211/71
GGA CAG GGT CTG GAA TGG ATT GGG GCT ATT TAT CCT GGA GAT GGT GAT ACT AGC TAC ACT
G   Q   G   L   E   W   I   G   A   I   Y   P   G   D   G   D   T   S   Y   T
241/81                                                  271/91
CAG AAG TTC AGG GGC AAG GCC ACA TTG ACT GCA GAT AAA TCC TCC AGC ACA GCC TAC ATG
Q   K   F   R   G   K   A   T   L   T   A   D   K   S   S   S   T   A   Y   M
301/101                                                 331/111
CAA CTC AGC AGC TTG GCA TCT GAG GAC TCT GCG GTC TAT TAC TGT GCA AGA CGT ACG GTA
Q   L   S   S   L   A   S   E   D   S   A   V   Y   Y   C   A   R   R   T   V
361/121                                                 391/131
GGA GGC TAC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC TCC ACC
G   G   Y   F   D   Y   W   G   Q   G   T   T   L   T   V   S   S   A   S   T
421/141                                                 451/151
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC
K   G   P   S   V   F   P   L   A   P   C   S   R   S   T   S   E   S   T   A
481/161                                                 511/171
GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
A   L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S
541/181                                                 571/191
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC
G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L   Y
601/201                                                 631/211
TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC
S   L   S   S   V   V   T   V   P   S   S   S   L   G   T   K   T   Y   T   C
661/221                                                 691/231
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT GAG TCC AAA TAT GGT
N   V   D   H   K   P   S   N   T   K   V   D   K   R   V   E   S   K   Y   G
721/241                                                 751/251
CCC CCA TGC CCA TCA TGC CCA GCA CCT GAG TTC CTG GGG GGA CCA TCA GTC TTC CTG TTC
P   P   C   P   S   C   P   A   P   E   F   L   G   G   P   S   V   F   L   F
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781/261
CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC
P P K P K D T L M I
811/271
TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG
S R T P E V T C V V
841/281
GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC
V D V S Q E D P E V
871/291
CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG
Q F N W Y V D G V E
901/301
GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG
V H N A K T K P R E
931/311
GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC
E Q F N S T Y R V V
961/321
AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG
S V L T V L H Q D W
991/331
CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC
L N G K E Y K C K V
1021/341
TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG
S N K G L P S S I E
1051/351
AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC
K T I S K A K G Q P
1081/361
CGA GAG CCA CAG GTG TAC ACC CTG CCC CCA
R E P Q V Y T L P P
1111/371
TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC
S Q E E M T K N Q V
1141/381
AGC CTG ACC TGC CTG GTC AAA GGC TTC TAC
S L T C L V K G F Y
1171/391
CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC
P S D I A V E W E S
1201/401
AAT GGG CAG CCG GAG AAC AAC TAC AAG ACC
N G Q P E N N Y K T
1231/411
ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC
T P P V L D S D G S
1261/421
TTC TTC CTC TAC AGC AGG CTA ACC GTG GAC
F F L Y S R L T V D
1291/431
AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC
K S R W Q E G N V F
1321/441
TCA TGC TCC GTG ATG CAT GAG GCT CTG CAC
S C S V M H E A L H
1351/451
AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG
N H Y T Q K S L S L
1381/461
TCT CTG GGT AAA TGA
S L G K *

3F4 (CHIMERIC) HUMAN G2/G4 CDNA

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1/1                                                 31/11
ATG AAG TGG AGC TGG GTT ATT CTC TTC CTC CTG TCA GTA ACT GCC GGC GTC CAC TCC CAG
M   K   W   S   W   V   I   L   F   L   L   S   V   T   A   G   V   H   S   Q
61/21                                               91/31
GTT CAG GTC CAG CAG TCT GGG GCT GAG CTG GCA AGA CCT TGG GCT TCA GTG AAG TTG TCC
V   Q   V   Q   Q   S   G   A   E   L   A   R   P   W   A   S   V   K   L   S
121/41                                              151/51
TGC AAG GCT TCT GGC TAC AAT TTT AAT AGT TAC TGG ATG CAG TGG GTA AAA CAG AGG CCT
C   K   A   S   G   Y   N   F   N   S   Y   W   M   Q   W   V   K   Q   R   P
181/61                                              211/71
GGA CAG GGT CTG GAA TGG ATT GGG GCT ATT TAT CCT GGA GAT GGT GAT ACT AGC TAC ACT
G   Q   G   L   E   W   I   G   A   I   Y   P   G   D   G   D   T   S   Y   T
241/81                                              271/91
CAG AAG TTC AGG GGC AAG GCC ACA TTG ACT GCA GAT AAA TCC TCC AGC ACA GCC TAC ATG
Q   K   F   R   G   K   A   T   L   T   A   D   K   S   S   S   T   A   Y   M
301/101                                             331/111
CAA CTC AGC AGC TTG GCA TCT GAG GAC TCT GCG GTC TAT TAC TGT GCA AGA CGT ACG GTA
Q   L   S   S   L   A   S   E   D   S   A   V   Y   Y   C   A   R   R   T   V
361/121                                             391/131
GGA GGC TAC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC TCC ACC
G   G   Y   F   D   Y   W   G   Q   G   T   T   L   T   V   S   S   A   S   T
421/141                                             451/151
AAG GGC CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC
K   G   P   S   V   F   P   L   A   P   C   S   R   S   T   S   E   S   T   A
481/161                                             511/171
GCC CTG GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA
A   L   G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S
541/181                                             571/191
GGC GCC CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC
G   A   L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L   Y
601/201                                             631/211
TCC CTC AGC AGC GTG GTG ACC GTG CCC TCC AGC AAC TTC GGC ACC CAG ACC TAC ACC TGC
S   L   S   S   V   V   T   V   P   S   S   N   F   G   T   Q   T   Y   T   C
661/221                                             691/231
AAC GTA GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG ACA GTT GAG CGC AAA TGT TGT
N   V   D   H   K   P   S   N   T   K   V   D   K   T   V   E   R   K   C   C
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721/241                                                 751/251
GTC GAG TGC CCA CCG TGC CCA GCA CCA CCT GTG GCA GGA CCG TCA GTC TTC CTC TTC CCC
V   E   C   P   P   C   P   A   P   P   V   A   G   P   S   V   F   L   F   P
781/261                                                 811/271
CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT GAG GTC ACG TGC GTG GTG GTG
P   K   P   K   D   T   L   M   I   S   R   T   P   E   V   T   C   V   V   V
841/281                                                 871/291
GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG TAC GTG GAT GGC GTG GAG GTG
D   V   S   Q   E   D   P   E   V   Q   F   N   W   Y   V   D   G   V   E   V
901/301                                                 931/311
CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC AGC ACG TAC CGT GTG GTC AGC
H   N   A   K   T   K   P   R   E   E   Q   F   N   S   T   Y   R   V   V   S
961/321                                                 991/331
GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG GAG TAC AAG TGC AAG GTC TCC
V   L   T   V   L   H   Q   D   W   L   N   G   K   E   Y   K   C   K   V   S
1021/341                                                1051/351
AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC AAA GCC AAA GGG CAG CCC CGA
N   K   G   L   P   S   S   I   E   K   T   I   S   K   A   K   G   Q   P   R
1081/361                                                1111/371
GAG CCA CAG GTG TAC ACC CTG CCC CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC
E   P   Q   V   Y   T   L   P   P   S   Q   E   E   M   T   K   N   Q   V   S
1141/381                                                1171/391
CTG ACC TGC CTG GTC AAA GGC TTC TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT
L   T   C   L   V   K   G   F   Y   P   S   D   I   A   V   E   W   E   S   N
1201/401                                                1231/411
GGG CAG CCG GAG AAC AAC TAC AAG ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC
G   Q   P   E   N   N   Y   K   T   T   P   P   V   L   D   S   D   G   S   F
1261/421                                                1291/431
TTC CTC TAC AGC AGG CTA ACC GTG GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA
F   L   Y   S   R   L   T   V   D   K   S   R   W   Q   E   G   N   V   F   S
1321/441                                                1351/451
TGC TCC GTG ATG CAT GAG GCT CTG CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT
C   S   V   M   H   E   A   L   H   N   H   Y   T   Q   K   S   L   S   L   S
1381/461
CTG GGT AAA TGA
L   G   K   *
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3F4 HUMAN G2/G4 EXPRESSION PLASMID INSERT SEQUENCE

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   1 gtgaccaatacaaaacaaaagcgcccctcgtaccagcgaagaaggggcagagatgccgtagtcaggtttagttcgtccgg  80
  81 cggcgggggatctgtatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatctgctccctgctt 160
 161 gtgtgttggaggtcgctgagtagtgcgcgagcaaaatttaagctacaacaaggcaaggcttgaccgacaattgcatgaag 240
 241 aatctgcttagggttaggcgttttgcgctgcttcgcgatgtacgggccagatatacgcgttgacattgattattgactag 320
 321 ttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatg 400
 401 gcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaataggg 480
 481 actttccattgacgtcaatgggtggactatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaag 560
 561 tacgcccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctа 640
 641 cttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatag 720
 721 cggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacggga 800
 801 ctttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagca 880
 881 gagctcgtttagtgaaccgtcaGAATTCTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTG 960
 961 GATCGGAAACCCGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAAC 1040
1041 CTCTCGACTGTTGGGgtgagtactccctctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgag 1120
1121 gaggatttgatattcacctggcccgcggtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatcttttt 1200
1201 gttgtcaagcttgaggtgtggcaggcttgagatctggccatacacttgagtgacaatgacatccactttgcctttctctc 1280
1281 cacagGTGTCCACTCCCAGGTCCAACTGCAGGTCGACCGGCTTGGTACCGAGCTCGGATCCGGACCATC ATG AAG    1355
   1                                                                        M   K      2
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1356 TGG AGC TGG GTT ATT CTC TTC CTC CTG TCA GTA ACT GCC GGC GTC CAC TCC CAG GTT CAG  1415
   3 W   S   W   V   I   L   F   L   L   S   V   T   A   G   V   H   S   Q   V   Q    22

1416 GTC CAG CAG TCT GGG GCT GAG CTG GCA AGA CCT TGG GCT TCA GTG AAG TTG TCC TGC AAG  1475
  23 V   Q   Q   S   G   A   E   L   A   R   P   W   A   S   V   K   L   S   C   K    42

1476 GCT TCT GGC TAC AAT TTT AAT AGT TAC TGG ATG CAG TGG GTA AAA CAG AGG CCT GGA CAG  1535
  43 A   S   G   Y   N   F   N   S   Y   W   M   Q   W   V   K   Q   R   P   G   Q    62

1536 GGT CTG GAA TGG ATT GGG GCT ATT TAT CCT GGA GAT GGT GAT ACT AGC TAC ACT CAG AAG  1595
  63 G   L   E   W   I   G   A   I   Y   P   G   D   G   D   T   S   Y   T   Q   K    82

1596 TTC AGG GGC AAG GCC ACA TTG ACT GCA GAT AAA TCC TCC AGC ACA GCC TAC ATG CAA CTC  1655
  83 F   R   G   K   A   T   L   T   A   D   K   S   S   S   T   A   Y   M   Q   L   102

1656 AGC AGC TTG GCA TCT GAG GAC TCT GCG GTC TAT TAC TGT GCA AGA CGT ACG GTA GGA GGC  1715
 103 S   S   L   A   S   E   D   S   A   V   Y   Y   C   A   R   R   T   V   G   G   122

1716 TAC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC TCC ACC AAG GGC  1775
 123 Y   F   D   Y   W   G   Q   G   T   T   L   T   V   S   S   A   S   T   K   G   142

1776 CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC GCC CTG  1835
 143 P   S   V   F   P   L   A   P   C   S   R   S   T   S   E   S   T   A   A   L   162

1836 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC  1895
 163 G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S   G   A   182

1896 CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC  1955
 183 L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L   Y   S   L   202

1956 AGC AGC GTG GTG ACC GTG CCC TCC AGC AAC TTC GGC ACC CAG ACC TAC ACC TGC AAC GTA  2015
 203 S   S   V   V   T   V   P   S   S   N   F   G   T   Q   T   Y   T   C   N   V   222

2016 GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG ACA GTT G gtgagaggccagctcagggagggagg 2081
 223 D   H   K   P   S   N   T   K   V   D   K   T   V   E                           236

2082 gtgtctgctggaagccaggctcagccctcctgcctggacgcaccccggctgtgcagccccagcccagggcagcaaggcag 2161
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```
2162 gccccatctgtctcctcacccggaggcctctgcccgccccactcatgctcagggagagggtcttctggctttttccacca 2241

2242 ggctccaggcaggcacaggctgggtgcccctaccccaggcccttcacacacaggggcaggtgcttggctcagacctgcca 2321

2322 aaagccatatccgggaggaccctgcccctgacctaagccgaccccaaaggccaaactgtccactccctcagctcggacac 2401

2402 cttctctcctcccagatccgagtaactcccaatcttctctctgcag AG CGC AAA TGT TGT GTC GAG TGC    2470
 237                                                R   K   C   C   V   E   C      243

2471 CCA CCG TGC CCA G gtaagccagcccaggcctcgccctccagctcaaggcgggacaggtgccctagagtagcctgc 2545
 244 P   P   C   P   A                                                              248

2546 atccagggacaggccccagctgggtgctgacacgtccacctccatctcttcctcag CA CCA CCT GTG GCA GGA  2618
 249                                                          P   P   V   A   G    253

2619 CCG TCA GTC TTC CTC TTC CCC CCA AAA CCC AAG GAC ACC CTC ATG ATC TCC CGG ACC CCT  2678
 254 P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P    273

2679 GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG  2738
 274 E   V   T   C   V   V   V   D   V   S   Q   E   D   P   E   V   Q   F   N   W    293

2739 TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC  2798
 294 Y   V   D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q   F   N    313

2799 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG  2858
 314 S   T   Y   R   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K    333

2859 GAG TAC AAG TGC AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC  2918
 334 E   Y   K   C   K   V   S   N   K   G   L   P   S   S   I   E   K   T   I   S    353

2919 AAA GCC AAA G gtgggacccacggggtgcgagggccacacggacagaggccagctcggcccaccctctgccctggga 2994
 354 K   A   K   G                                                                  357

2995 gtgaccgctgtgccaacctctgtccctacag GG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC   3060
 358                                    Q   P   R   E   P   Q   V   Y   T   L   P     368

3061 CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC  3120
 369 P   S   Q   E   E   M   T   K   N   Q   V   S   L   T   C   L   V   K   G   F    388
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3121 TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG  3180
 389 Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K    408

3181 ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG  3240
 409 T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   R   L   T   V    428

3241 GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG  3300
 429 D   K   S   R   W   Q   E   G   N   V   F   S   C   S   V   M   H   E   A   L    448

3301 CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA TGA gtgccagggccattga 3364
 449 H   N   H   Y   T   Q   K   S   L   S   L   S   L   G   K   *                   464

3365 agcatttatcagggttattgtctcatgagcggatac                                          3400
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3F4 HUMAN IgG4 EXPRESSION PLASMID INSERT SEQUENCE

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   1 gtgaccaatacaaaacaaaagcgcccctcgtaccagcgaagaaggggcagagatgccgtagtcaggtttagttcgtccgg  80

  81 cggcgggggatctgtatggtgcactctcagtacaatctgctctgatgccgcatagttaagccagtatctgctccctgctt 160

 161 gtgtgttggaggtcgctgagtagtgcgcgagcaaaatttaagctacaacaaggcaaggcttgaccgacaattgcatgaag 240

 241 aatctgcttagggttaggcgttttgcgctgcttcgcgatgtacgggccagatatacgcgttgacattgattattgactag 320

 321 ttattaatagtaatcaattacggggtcattagttcatagcccatatatggagttccgcgttacataacttacggtaaatg 400

 401 gcccgcctggctgaccgcccaacgacccccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaataggg 480

 481 actttccattgacgtcaatgggtggactatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaag 560

 561 tacgccccctattgacgtcaatgacggtaaatggcccgcctggcattatgcccagtacatgaccttatgggactttccta 640

 641 cttggcagtacatctacgtattagtcatcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatag 720

 721 cggtttgactcacggggatttccaagtctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacggga 800
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 801 ctttccaaaatgtcgtaacaactccgccccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagca 880

 881 gagctcgtttagtgaaccgtcaGAATTCTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTG 960

 961 GATCGGAAACCCGTCGGCCTCCGAACGGTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAAC 1040

1041 CTCTCGACTGTTGGGgtgagtactccctctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgag 1120

1121 gaggatttgatattcacctggcccgcggtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatcttttt 1200

1201 gttgtcaagcttgaggtgtggcaggcttgagatctggccatacacttgagtgacaatgacatccactttgcctttctctc 1280

1281 cacagGTGTCCACTCCCAGGTCCAACTGCAGGTCGACCGGCTTGGTACCGAGCTCGGATCCGGACCATC ATG AAG    1355
   1                                                                      M   K      2

1356 TGG AGC TGG GTT ATT CTC TTC CTC CTG TCA GTA ACT GCC GGC GTC CAC TCC CAG GTT CAG  1415
   3 W   S   W   V   I   L   F   L   L   S   V   T   A   G   V   H   S   Q   V   Q    22

1416 GTC CAG CAG TCT GGG GCT GAG CTG GCA AGA CCT TGG GCT TCA GTG AAG TTG TCC TGC AAG  1475
  23 V   Q   Q   S   G   A   E   L   A   R   P   W   A   S   V   K   L   S   C   K    42

1476 GCT TCT GGC TAC AAT TTT AAT AGT TAC TGG ATG CAG TGG GTA AAA CAG AGG CCT GGA CAG  1535
  43 A   S   G   Y   N   F   N   S   Y   W   M   Q   W   V   K   Q   R   P   G   Q    62

1536 GGT CTG GAA TGG ATT GGG GCT ATT TAT CCT GGA GAT GGT GAT ACT AGC TAC ACT CAG AAG  1595
  63 G   L   E   W   I   G   A   I   Y   P   G   D   G   D   T   S   Y   T   Q   K    82

1596 TTC AGG GGC AAG GCC ACA TTG ACT GCA GAT AAA TCC TCC AGC ACA GCC TAC ATG CAA CTC  1655
  83 F   R   G   K   A   T   L   T   A   D   K   S   S   S   T   A   Y   M   Q   L   102

1656 AGC AGC TTG GCA TCT GAG GAC TCT GCG GTC TAT TAC TGT GCA AGA CGT ACG GTA GGA GGC  1715
 103 S   S   L   A   S   E   D   S   A   V   Y   Y   C   A   R   R   T   V   G   G   122

1716 TAC TTT GAC TAC TGG GGC CAA GGC ACC ACT CTC ACA GTC TCC TCA GCC TCC ACC AAG GGC  1775
 123 Y   F   D   Y   W   G   Q   G   T   T   L   T   V   S   S   A   S   T   K   G   142
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1776 CCA TCC GTC TTC CCC CTG GCG CCC TGC TCC AGG AGC ACC TCC GAG AGC ACA GCC GCC CTG  1835
 143 P   S   V   F   P   L   A   P   C   S   R   S   T   S   E   S   T   A   A   L    162

1836 GGC TGC CTG GTC AAG GAC TAC TTC CCC GAA CCG GTG ACG GTG TCG TGG AAC TCA GGC GCC  1895
 163 G   C   L   V   K   D   Y   F   P   E   P   V   T   V   S   W   N   S   G   A    182

1896 CTG ACC AGC GGC GTG CAC ACC TTC CCG GCT GTC CTA CAG TCC TCA GGA CTC TAC TCC CTC  1955
 183 L   T   S   G   V   H   T   F   P   A   V   L   Q   S   S   G   L   Y   S   L    202

1956 AGC AGC GTG GTG ACC GTG CCC TCC AGC AGC TTG GGC ACG AAG ACC TAC ACC TGC AAC GTA  2015
 203 S   S   V   V   T   V   P   S   S   S   L   G   T   K   T   Y   T   C   N   V    222

2016 GAT CAC AAG CCC AGC AAC ACC AAG GTG GAC AAG AGA GTT G gtgagaggccagcacagggagggagg 2081
 223 D   H   K   P   S   N   T   K   V   D   K   R   V   E                           236

2082 gtgtctgctggaagccaggctcagccctcctgcctggacgcaccccggctgtgcagccccagcccagggcagcaaggcat 2161

2162 gccccatctgtctcctcacccggaggcctctgaccaccccactcatgctcagggagagggtcttctggatttttccacca 2241

2242 ggctcccggcaccacaggctggatgcccctaccccaggccctgcgcatacagggcaggtgctgcgctcagacctgccaag 2321

2322 agccatatccgggaggaccctgcccctgacctaagcccaccccaaaggccaaactctccactccctcagctcagacacct 2401

2402 tctctcctcccagatctgagtaactcccaatcttctctctgcag AG TCC AAA TAT GGT CCC CCA TGC CCA  2471
 237                                              S   K   Y   G   P   P   C   P    244

2472 TCA TGC CCA G gtaagccaacccaggcctcgccctccagctcaaggcgggacaggtgccctagagtagcctgcatcc 2547
 245 S   C   P   A                                                                   248

2548 agggacaggccccagccgggtgctgacgcatccacctccatctcttcctcag CA CCT GAG TTC CTG GGG GGA  2619
 249                                                      P   E   F   L   G   G    254

2620 CCA TCA GTC TTC CTG TTC CCC CCA AAA CCC AAG GAC ACT CTC ATG ATC TCC CGG ACC CCT  2679
 255 P   S   V   F   L   F   P   P   K   P   K   D   T   L   M   I   S   R   T   P    274

2680 GAG GTC ACG TGC GTG GTG GTG GAC GTG AGC CAG GAA GAC CCC GAG GTC CAG TTC AAC TGG  2739
 275 E   V   T   C   V   V   V   D   V   S   Q   E   D   P   E   V   Q   F   N   W    294
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2740 TAC GTG GAT GGC GTG GAG GTG CAT AAT GCC AAG ACA AAG CCG CGG GAG GAG CAG TTC AAC  2799
 295 Y   V   D   G   V   E   V   H   N   A   K   T   K   P   R   E   E   Q   F   N    314

2800 AGC ACG TAC CGT GTG GTC AGC GTC CTC ACC GTC CTG CAC CAG GAC TGG CTG AAC GGC AAG  2859
 315 S   T   Y   R   V   V   S   V   L   T   V   L   H   Q   D   W   L   N   G   K    334

2860 GAG TAC AAG TGC AAG GTC TCC AAC AAA GGC CTC CCG TCC TCC ATC GAG AAA ACC ATC TCC  2919
 335 E   Y   K   C   K   V   S   N   K   G   L   P   S   S   I   E   K   T   I   S    354

2920 AAA GCC AAA G gtgggacccacggggtgcgagggccacacggacagaggccagctcggcccaccctctgccctggga 2995
 355 K   A   K   G                                                                    358

2996 gtgaccgctgtgccaacctctgtccctacag GG CAG CCC CGA GAG CCA CAG GTG TAC ACC CTG CCC   3061
 359                                   Q   P   R   E   P   Q   V   Y   T   L   P     369

3062 CCA TCC CAG GAG GAG ATG ACC AAG AAC CAG GTC AGC CTG ACC TGC CTG GTC AAA GGC TTC  3121
 370 P   S   Q   E   E   M   T   K   N   Q   V   S   L   T   C   L   V   K   G   F    389

3122 TAC CCC AGC GAC ATC GCC GTG GAG TGG GAG AGC AAT GGG CAG CCG GAG AAC AAC TAC AAG  3181
 390 Y   P   S   D   I   A   V   E   W   E   S   N   G   Q   P   E   N   N   Y   K    409

3182 ACC ACG CCT CCC GTG CTG GAC TCC GAC GGC TCC TTC TTC CTC TAC AGC AGG CTA ACC GTG  3241
 410 T   T   P   P   V   L   D   S   D   G   S   F   F   L   Y   S   R   L   T   V    429

3242 GAC AAG AGC AGG TGG CAG GAG GGG AAT GTC TTC TCA TGC TCC GTG ATG CAT GAG GCT CTG  3301
 430 D   K   S   R   W   Q   E   G   N   V   F   S   C   S   V   M   H   E   A   L    449

3302 CAC AAC CAC TAC ACA CAG AAG AGC CTC TCC CTG TCT CTG GGT AAA TGA gtgccagggccattga 3365
 450 H   N   H   Y   T   Q   K   S   L   S   L   S   L   G   K   *                   465

3366 agcatttatcagggttattgtctcatgagcggatacatatttgaatgtatttagaaaaataaacaaataggggttccgcg 3445

3446 cacatttccccgaaaagtgccacctgacgcgttgacattgattattgactagttattaatagtaatcaattacggggtca 3525

3526 ttagttcatagcccatatatggagttccgcgttacataacttacggtaaatggccccgcctggctgaccgcccaacgacc 3605

3606 cccgcccattgacgtcaataatgacgtatgttcccatagtaacgccaatagggactttccattgacgtcaatgggtggac 3685
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3686 tatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacgg 3765

3766 taaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtca 3845

3846 tcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacggggatttccaagt 3925

3926 ctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgc 4005

4006 cccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgtttagtgaaccgtcaGAATT 4085

4086 CTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAACG 4165

4166 GTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAACCTCTCGACTGTTGGGgtgagtactccc 4245

4246 tctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgaggaggatttgatattcacctggcccgcg 4325

4326 gtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatctttttgttgtcaagcttgaggtgtggcaggct 4405

4406 tgagatctggccatacacttgagtgacaatgacatccactttgcctttctctccacagGTGTCCACTCCCAGGTCCAACT 4485

4486 GCAGGTCGAC ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GTT TCC   4546
   1           M   K   L   P   V   R   L   L   V   L   M   F   W   I   P   V   S    17

4547 AGC AGT GAT GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA  4606
  18 S   S   D   V   V   M   T   Q   T   P   L   S   L   P   V   S   L   G   D   Q   37

4607 GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC CTT GTA CAC AGT AAT GGA AAC ACC TAT TTA  4666
  38 A   S   I   S   C   R   S   S   Q   S   L   V   H   S   N   G   N   T   Y   L   57

4667 CAG TGG TAC CTG CAG AAG CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC  4726
  58 Q   W   Y   L   Q   K   P   G   Q   S   P   K   L   L   I   Y   K   V   S   N   77

4727 CGA TTT TCT GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC  4786
  78 R   F   S   G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T   L   97

4787 AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TTC TGT TCT CAA AGT ACA CAT  4846
  98 K   I   S   R   V   E   A   E   D   L   G   V   Y   F   C   S   Q   S   T   H  117
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3686 tatttacggtaaactgcccacttggcagtacatcaagtgtatcatatgccaagtacgccccctattgacgtcaatgacgg 3765

3766 taaatggcccgcctggcattatgcccagtacatgaccttatgggactttcctacttggcagtacatctacgtattagtca 3845

3846 tcgctattaccatggtgatgcggttttggcagtacatcaatgggcgtggatagcggtttgactcacggggatttccaagt 3925

3926 ctccaccccattgacgtcaatgggagtttgttttggcaccaaaatcaacgggactttccaaaatgtcgtaacaactccgc 4005

4006 cccattgacgcaaatgggcggtaggcgtgtacggtgggaggtctatataagcagagctcgtttagtgaaccgtcaGAATT 4085

4086 CTGTTGGGCTCGCGGTTGATTACAAACTCTTCGCGGTCTTTCCAGTACTCTTGGATCGGAAACCCGTCGGCCTCCGAACG 4165

4166 GTACTCCGCCACCGAGGGACCTGAGCGAGTCCGCATCGACCGGATCGGAAAACCTCTCGACTGTTGGGgtgagtactccc 4245

4246 tctcaaaagcgggcatgacttctgcgctaagattgtcagtttccaaaaacgaggaggatttgatattcacctggcccgcg 4325

4326 gtgatgcctttgagggtggccgcgtccatctggtcagaaaagacaatctttttgttgtcaagcttgaggtgtggcaggct 4405

4406 tgagatctggccatacacttgagtgacaatgacatccactttgcctttctctccacagGTGTCCACTCCCAGGTCCAACT 4485

4486 GCAGGTCGAC ATG AAG TTG CCT GTT AGG CTG TTG GTG CTG ATG TTC TGG ATT CCT GTT TCC   4546
   1           M   K   L   P   V   R   L   L   V   L   M   F   W   I   P   V   S     17

4547 AGC AGT GAT GTT GTG ATG ACC CAA ACT CCA CTC TCC CTG CCT GTC AGT CTT GGA GAT CAA  4606
  18 S   S   D   V   V   M   T   Q   T   P   L   S   L   P   V   S   L   G   D   Q    37

4607 GCC TCC ATC TCT TGC AGA TCT AGT CAG AGC CTT GTA CAC AGT AAT GGA AAC ACC TAT TTA  4666
  38 A   S   I   S   C   R   S   S   Q   S   L   V   H   S   N   G   N   T   Y   L    57

4667 CAG TGG TAC CTG CAG AAG CCA GGC CAG TCT CCA AAG CTC CTG ATC TAC AAA GTT TCC AAC  4726
  58 Q   W   Y   L   Q   K   P   G   Q   S   P   K   L   L   I   Y   K   V   S   N    77

4727 CGA TTT TCT GGG GTC CCA GAC AGG TTC AGT GGC AGT GGA TCA GGG ACA GAT TTC ACA CTC  4786
  78 R   F   S   G   V   P   D   R   F   S   G   S   G   S   G   T   D   F   T   L    97

4787 AAG ATC AGC AGA GTG GAG GCT GAG GAT CTG GGA GTT TAT TTC TGT TCT CAA AGT ACA CAT  4846
  98 K   I   S   R   V   E   A   E   D   L   G   V   Y   F   C   S   Q   S   T   H   117
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4847 GTT CCG TTC ACG TTC GGA GGG GGG ACC AAG CTG GAA ATA AAA CGA ACT GTG GCT GCA CCA  4906
 118 V   P   F   T   F   G   G   G   T   K   L   E   I   K   R   T   V   A   A   P    137

4907 TCT GTC TTC ATC TTC CCG CCA TCT GAT GAG CAG TTG AAA TCT GGA ACT GCC TCT GTT GTG  4966
 138 S   V   F   I   F   P   P   S   D   E   Q   L   K   S   G   T   A   S   V   V    157

4967 TGC CTG CTG AAT AAC TTC TAT CCC AGA GAG GCC AAA GTA CAG TGG AAG GTG GAT AAC GCC  5026
 158 C   L   L   N   N   F   Y   P   R   E   A   K   V   Q   W   K   V   D   N   A    177

5027 CTC CAA TCG GGT AAC TCC CAG GAG AGT GTC ACA GAG CAG GAC AGC AAG GAC AGC ACC TAC  5086
 178 L   Q   S   G   N   S   Q   E   S   V   T   E   Q   D   S   K   D   S   T   Y    197

5087 AGC CTC AGC AGC ACC CTG ACG CTG AGC AAA GCA GAC TAC GAG AAA CAC AAA GTC TAC GCC  5146
 198 S   L   S   S   T   L   T   L   S   K   A   D   Y   E   K   H   K   V   Y   A    217

5147 TGC GAA GTC ACC CAT CAG GGC CTG AGC TCG CCC GTC ACA AAG AGC TTC AAC AGG GGA GAG  5206
 218 C   E   V   T   H   Q   G   L   S   S   P   V   T   K   S   F   N   R   G   E    237

5207 TGT TAG ctcgagcatgcaggcatgcaagcttggcactggccgtcgttttacaacgtcgtgactgggaaaaccctggcg 5284
 238 C   *                                                                            239

5285 ttacccaacttaatcg                                                                 5300
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Cloning of Porcine CD86 (B7-2) RT-PCR was used to amplify an internal segment of the porcine CD86 gene from RNA isolated from LPS stimulated porcine PBLs. A second PCR fragment encoding a truncated N-terminus was prepared using the same cDNA template and an anchor dependent 5' RACE PCR cloning kit (CLONTECH, San Diego, CA). These porcine PCR products were fused by overlapping PCR and ligated into a plasmid vector for sequencing.

The cloned portion of porcine CD86 comprises 577 nucleotides. the encoded polypeptide is 192 amino acids long. The partial gene fragment was subsequently fused to the carboxy terminal 49 amino acids of the human CD86 IgC domain by overlapping PCR in which the 5' primer was constructed so as to encode the first 4 N-terminal amino acid residues of human CD86. to facilitate efficient secretion from mammalian cells. The 3' primer included fifteen nucleotides encoding a 5 histidine tag sequence.

The sequence of the chimeric human/porcine CD86 is shown below. Amino acid residues 1-4 and 197-245 are from human CD86. Residues 1-25 are believed to encode a signal sequence. Primers used for cloning had sequences corresponding to (separately) nucleotides 166-184, nucleotides 574-595, nucleotides 1-33, nucleotides 585-764, and nucleotides 728-764. The porcine CD86 sequence of the invention spans nucleotides 19-597.

```
TCT AGA ATG GAT CCC CAG TGC ACT ATG GGA CTG AGA AAC ATT CTC GTT  48
        Met Asp Pro Gln Cys Thr Met Gly Leu Arg Asn Ile Leu Val
          1               5                  10

GGG ATG GTC CTC CTG CTC TCT GGT GCT GCC TCC TTG AAA AGT CAG GCA  96
Gly Met Val Leu Leu Leu Ser Gly Ala Ala Ser Leu Lys Ser Gln Ala
 15                  20                  25                  30

TAT TTC AAT GAG ACT GGA GAA CTG CCG TGC CAT TTT ACA AAC TCG CAG 144
Tyr Phe Asn Glu Thr Gly Glu Leu Pro Cys His Phe Thr Asn Ser Gln
                35                  40                  45

AAC CTA AGC CTG GAT GAG CTG GTA GTA TTT TGG CAG GAC CAG GAT AAC 192
Asn Leu Ser Leu Asp Glu Leu Val Val Phe Trp Gln Asp Gln Asp Asn
            50                  55                  60

CTG GTT CTC TAC GAG CTA TAC CGA GGC CAA GAG AAG CCT CAT AAT GTT 240
Leu Val Leu Tyr Glu Leu Tyr Arg Gly Gln Glu Lys Pro His Asn Val
        65                  70                  75

AAT TCC AAG TAT ATG GGT CGC ACA AGC TTT GAC CAG GCC ACC TGG ACC 288
Asn Ser Lys Tyr Met Gly Arg Thr Ser Phe Asp Gln Ala Thr Trp Thr
    80                  85                  90

CTG AGA CTC CAC AAC GTT CAA ATC AAG GAC AAG GGC TCA TAT CAA TGT 336
Leu Arg Leu His Asn Val Gln Ile Lys Asp Lys Gly Ser Tyr Gln Cys
 95                  100                 105                 110

TTC ATC CAT CAT AAA GGG CCG CAT GGA CTT GTT CCT ATC CAC CAG ATG 384
Phe Ile His His Lys Gly Pro His Gly Leu Val Pro Ile His Gln Met
                115                 120                 125

AGT TCT GAC CTA TCA GTG CTT GCT AAC TTC AGT CAA CCT GAA ATA AAC 432
Ser Ser Asp Leu Ser Val Leu Ala Asn Phe Ser Gln Pro Glu Ile Asn
            130                 135                 140
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CTA CTT ACT AAT CAC ACA GAA AAT TCT GTC ATA AAT TTG ACC TGC TCA 480
Leu Leu Thr Asn His Thr Glu Asn Ser Val Ile Asn Leu Thr Cys Ser
        145                 150                 155

TCT ACA CAA GGC TAC CCA GAA CCC CAG AGG ATG TAT ATG TTG CTA AAT 528
Ser Thr Gln Gly Tyr Pro Glu Pro Gln Arg Met Tyr Met Leu Leu Asn
    160                 165                 170

ACG AAG AAT TCA ACC ACT GAG CAT GAT GCT GAC ATG AAG AAA TCT CAA 576
Thr Lys Asn Ser Thr Thr Glu His Asp Ala Asp Met Lys Lys Ser Gln
175                 180                 185                 190

GAT AAT GTC ACA GAA CTG TAT GAC GTT TCC ATC AGC TTG TCT GTT TCA 624
Asp Asn Val Thr Glu Leu Tyr Asp Val Ser Ile Ser Leu Ser Val Ser
                195                 200                 205

TTC CCT GAT GTT ACG AGC AAT ATG ACC ATC TTC TGT ATT CTG GAA ACT 672
Phe Pro Asp Val Thr Ser Asn Met Thr Ile Phe Cys Ile Leu Glu Thr
            210                 215                 220

GAC AAG ACG CGG CTT TTA TCT TCA CCT TTC TCT ATA GAG CTT GAG GAC 720
Asp Lys Thr Arg Leu Leu Ser Ser Pro Phe Ser Ile Glu Leu Glu Asp
        225                 230                 235

CCT CAG CCT CCC CCA GAC CAC CAT CAC CAT CAC CAT TAA TGC AT      764
Pro Gln Pro Pro Pro Asp His His His His His His  *
    240                 245                 250
```

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What is claimed is:

1. An isolated antibody which binds to a porcine cell interaction protein selected from the group consisting of P-selectin, VCAM, and CD86 but not to a human cell interaction protein selected from the group consisting of P-selectin, VCAM, and CD86.

2. A method for treating rejection of a xenografted porcine organ, tissue, or cell comprising administering the antibody of Claim 1 to said organ, tissue, or cell.

3. A method for detecting rejection of a porcine organ, tissue, or cell that has been xenografted into a patient comprising assaying a body fluid of the patient for the presence of an antigen immunoreactive with the antibody of Claim 1.

4. The method of Claim 3 in which the body fluid is blood.

5. The isolated antibody of Claim 1 wherein the antibody is a recombinant antibody and comprises a chain coded for by a sequence selected from the sequences of pages 59-79.

6. An isolated nucleic acid molecule comprising:

(a) a sequence selected from the sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, and SEQ ID NO:14;

(b) a sequence complementary to (a); or

(c) both (a) and (b);

said molecule being substantially free of nucleic acid molecules not containing (a), (b), or (c).

7. An isolated nucleic acid molecule comprising:

(a) any of the CDR encoding regions of the antibody sequences of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:10, SEQ ID NO:11, or SEQ ID NO:12,; or

(b) a sequence complementary to (a); or

(c) both (a) and (b);

said molecule being substantially free of nucleic acid molecules not containing (a), (b), or (c).

8. An antibody comprising the C1 and hinge regions of human IgG2 and the C2 and C3 regions of human IgG4

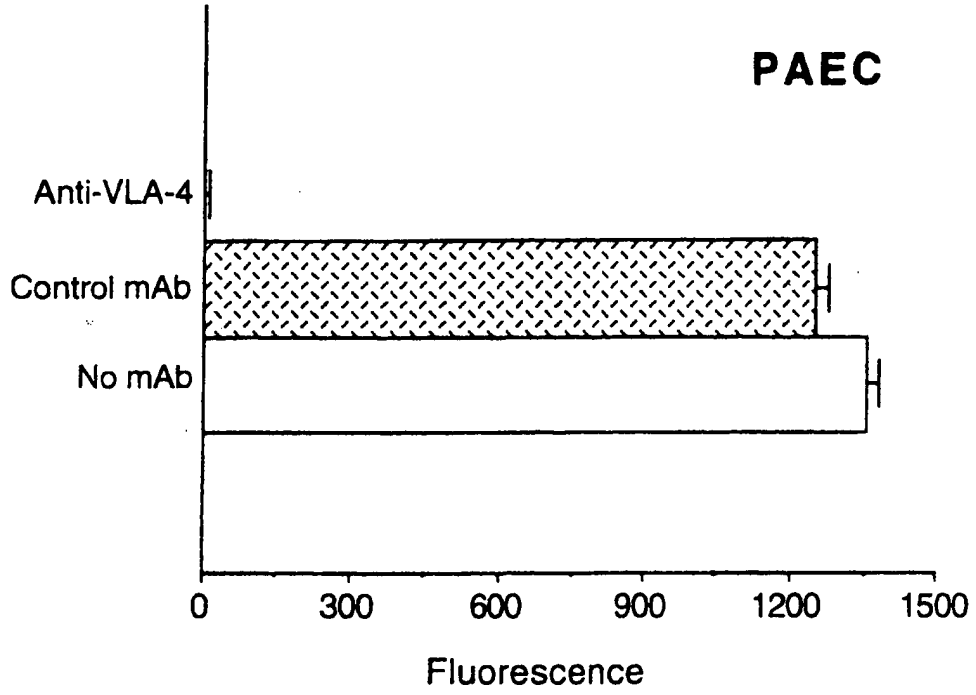

PAEC
Anti-VLA-4
Control mAb
No mAb
0
300
600
900
1200
1500
Fluorescence

Fig. 1a

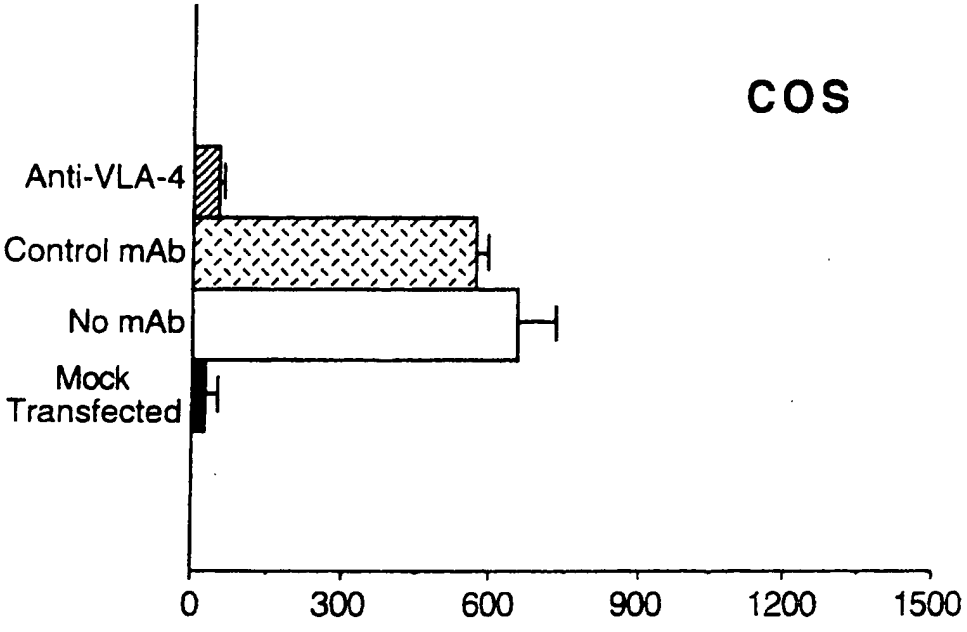

COS
Anti-VLA-4
Control mAb
No mAb
Mock Transfected
0
300
600
900
1200
1500
Fluorescence

Fig. 1b

Fig. 2A

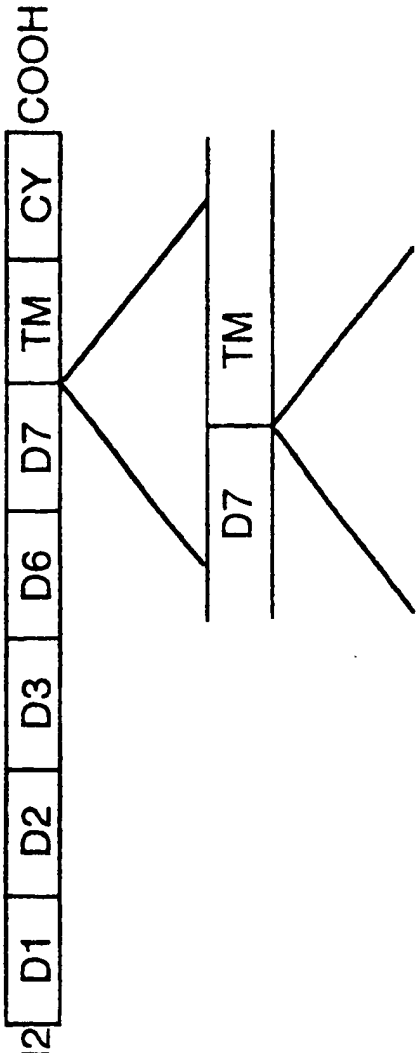
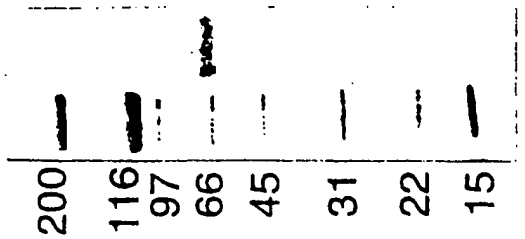

NH2
D1
D2
D3
D6
D7
TM
CY
COOH
D7
TM
Wild type TCTTCTGAACTTCTCGTGCTCTATTGT
S S E L L V L Y C
NsiI
spVCAM TCTTCTGAACACCATCACCATCACCATTAATGCAT
S S E H H H H H H *

Fig. 2B


200
116
97
66
45
31
22
15

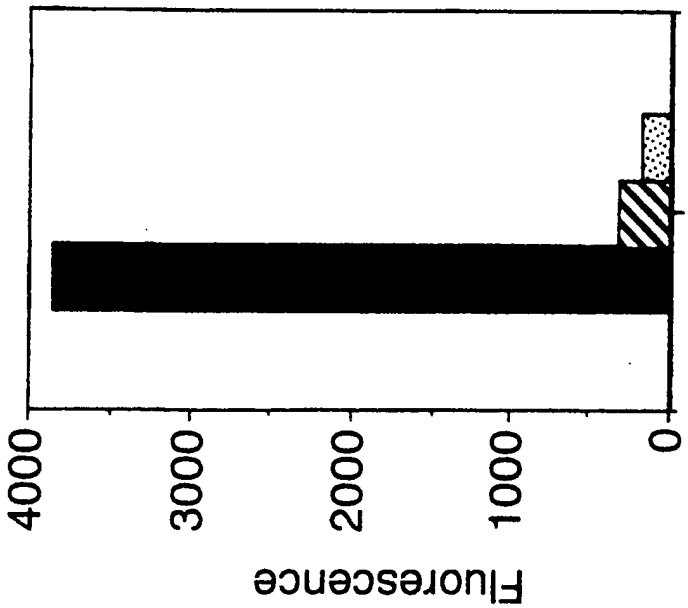

Fluorescence
4000
3000
2000
1000
0
no mAb
BSA
HP2/1

Fig. 3b

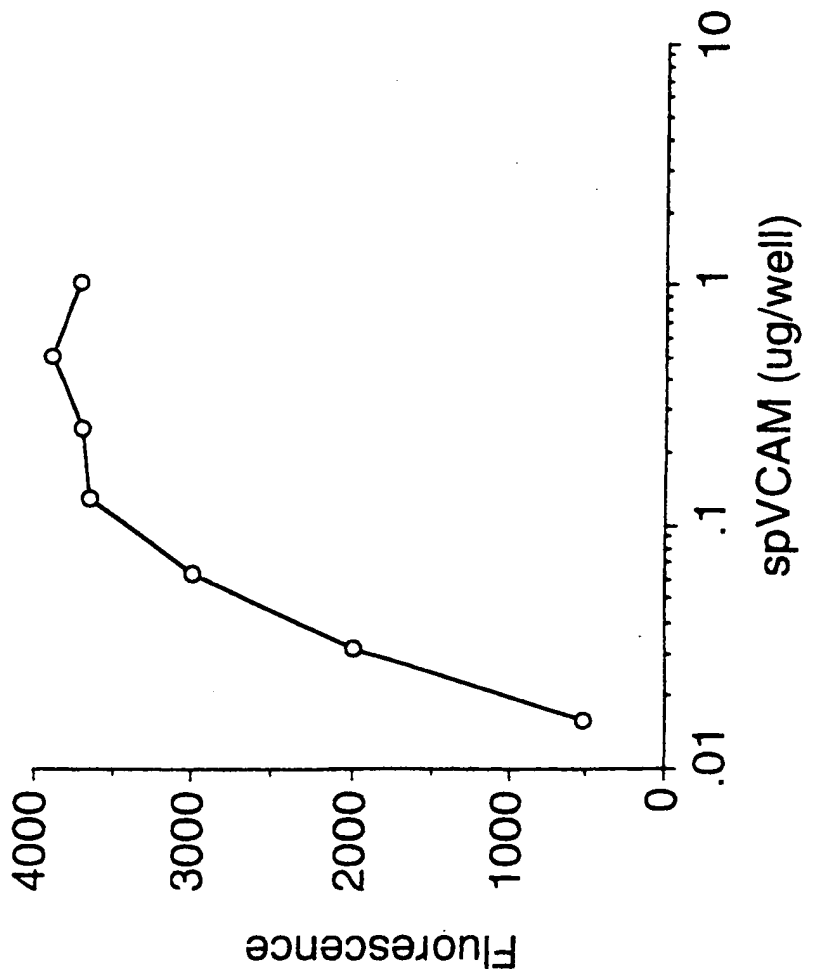

Fluorescence
4000
3000
2000
1000
0
.01
.1
1
10
spVCAM (ug/well)

Fig. 3a

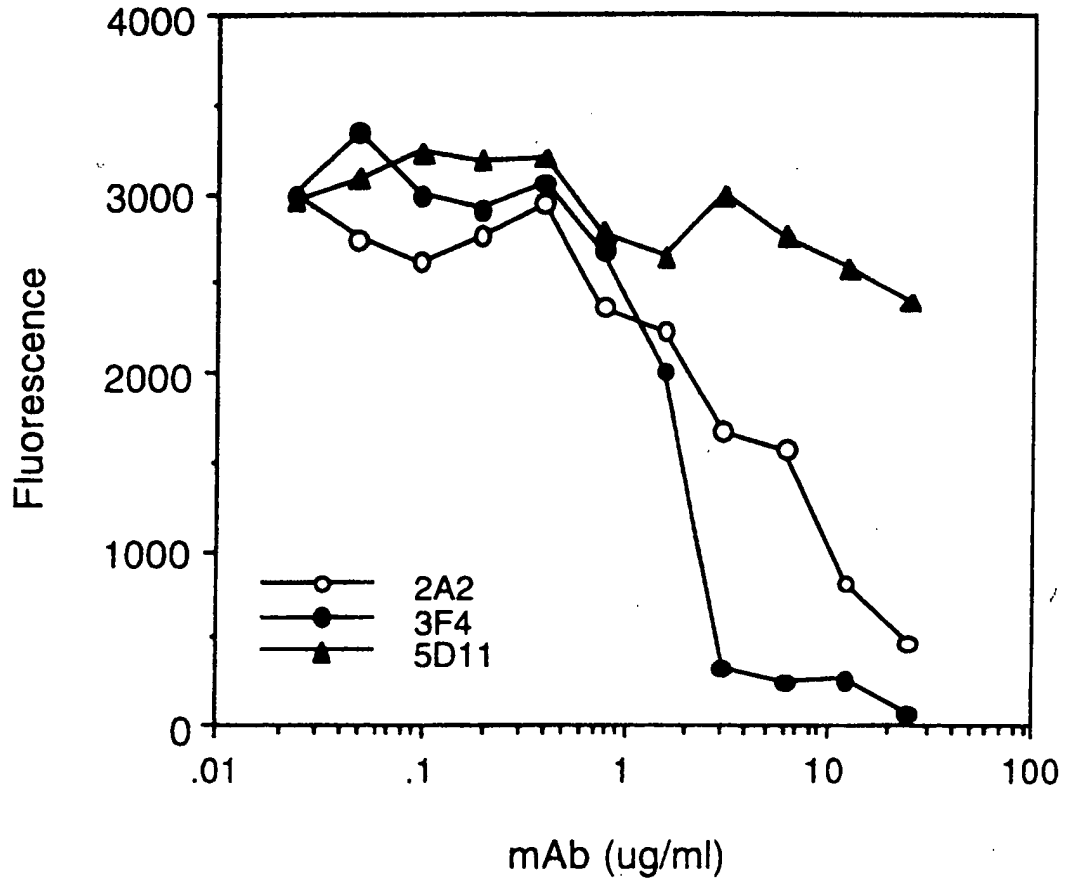

4000
3000
2000
1000
0
Fluorescence
.01
.1
1
10
100
mAb (ug/ml)
2A2
3F4
5D11

Fig. 4

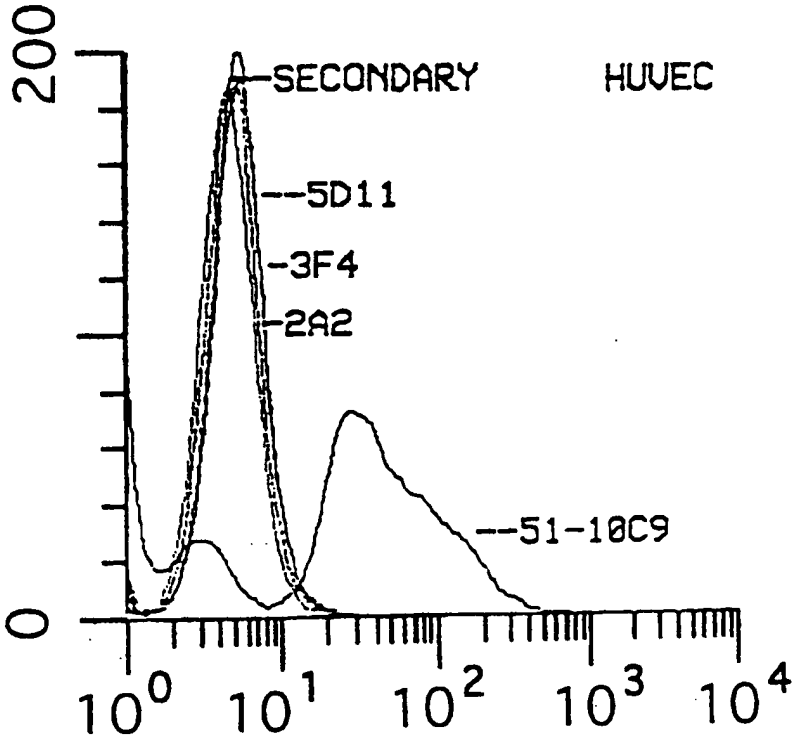
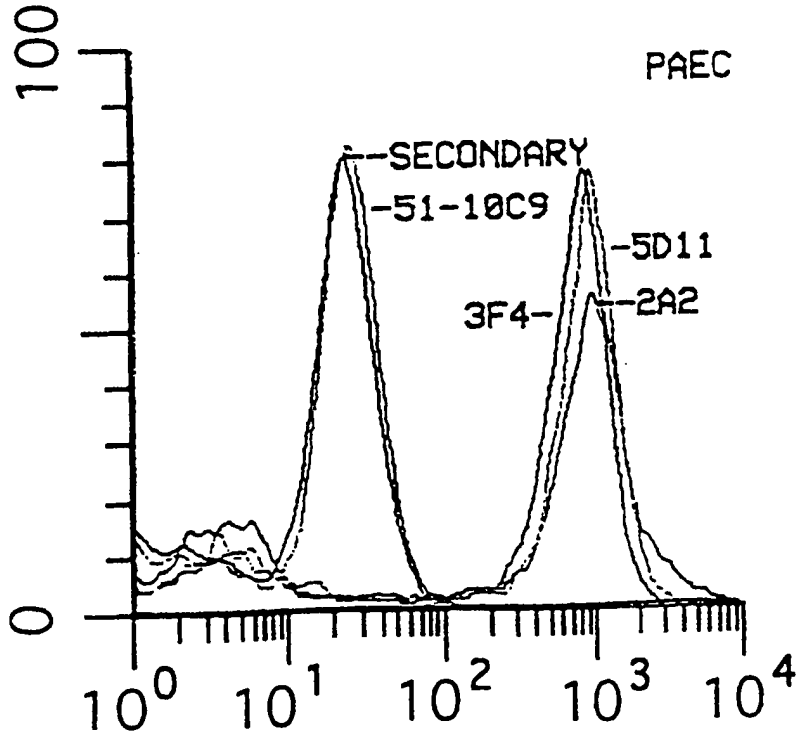

200
0
SECONDARY
HUVEC
5D11
3F4
2A2
51-10C9
10^0 10^1 10^2 10^3 10^4

Fig. 5a


100
0
PAEC
SECONDARY
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5D11
3F4
2A2
10^0 10^1 10^2 10^3 10^4

Fig 5b

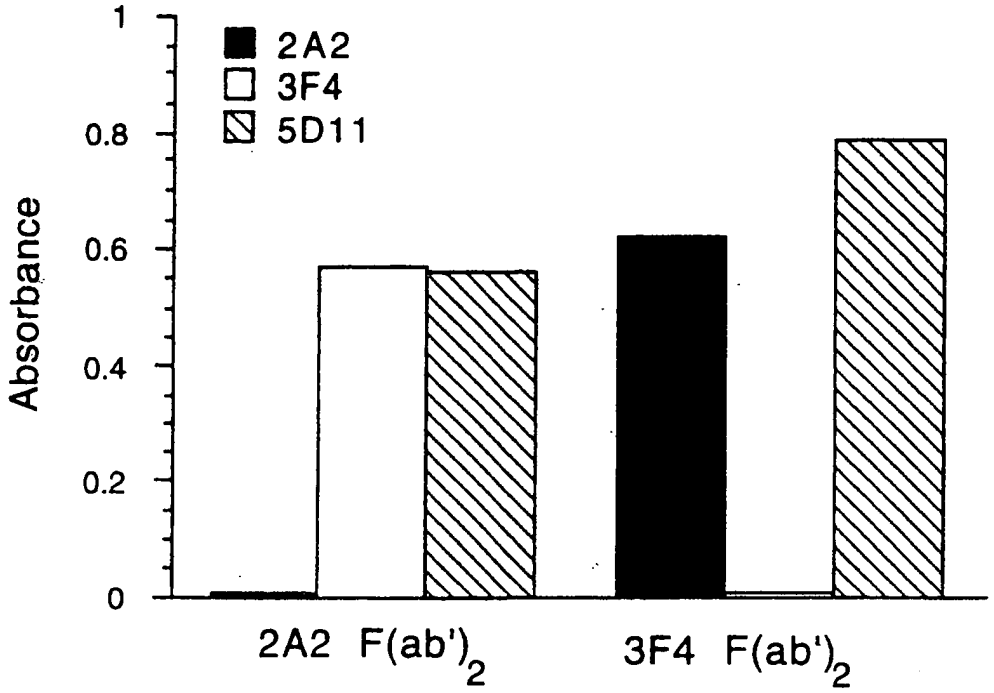

Fig. 6

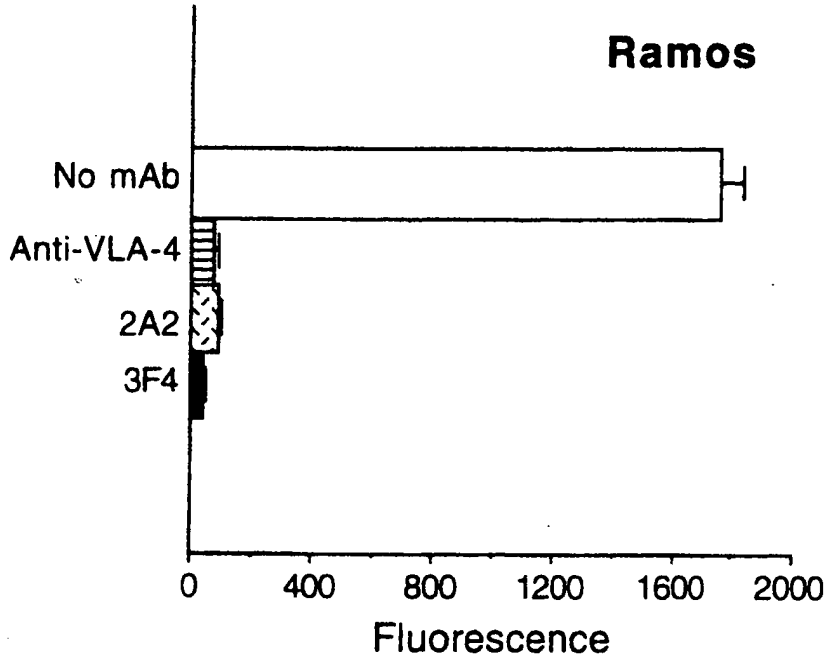
Ramos
No mAb
Anti-VLA-4
2A2
3F4
0
400
800
1200
1600
2000
Fluorescence

Fig. 7a

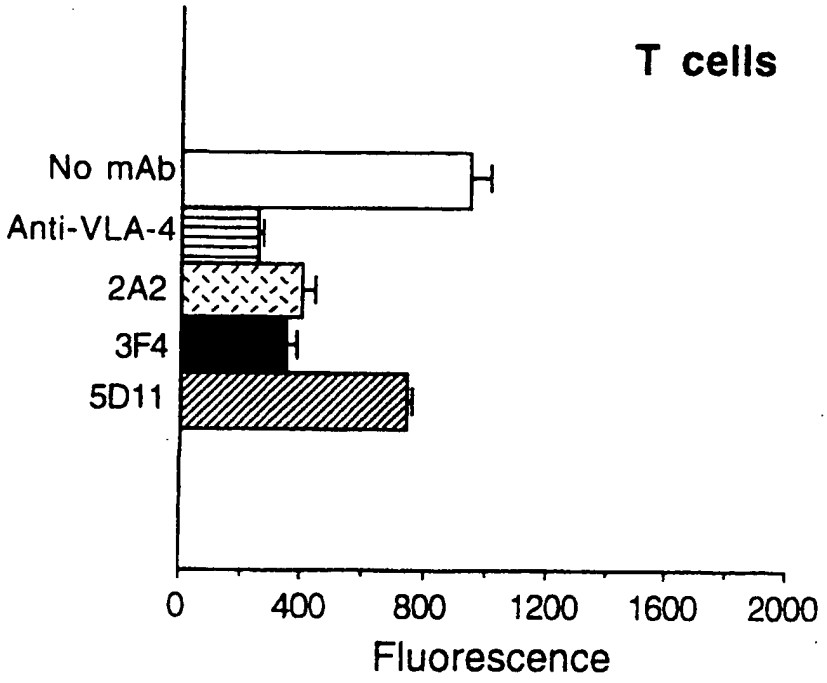
T cells
No mAb
Anti-VLA-4
2A2
3F4
5D11
0
400
800
1200
1600
2000
Fluorescence

Fig. 7b

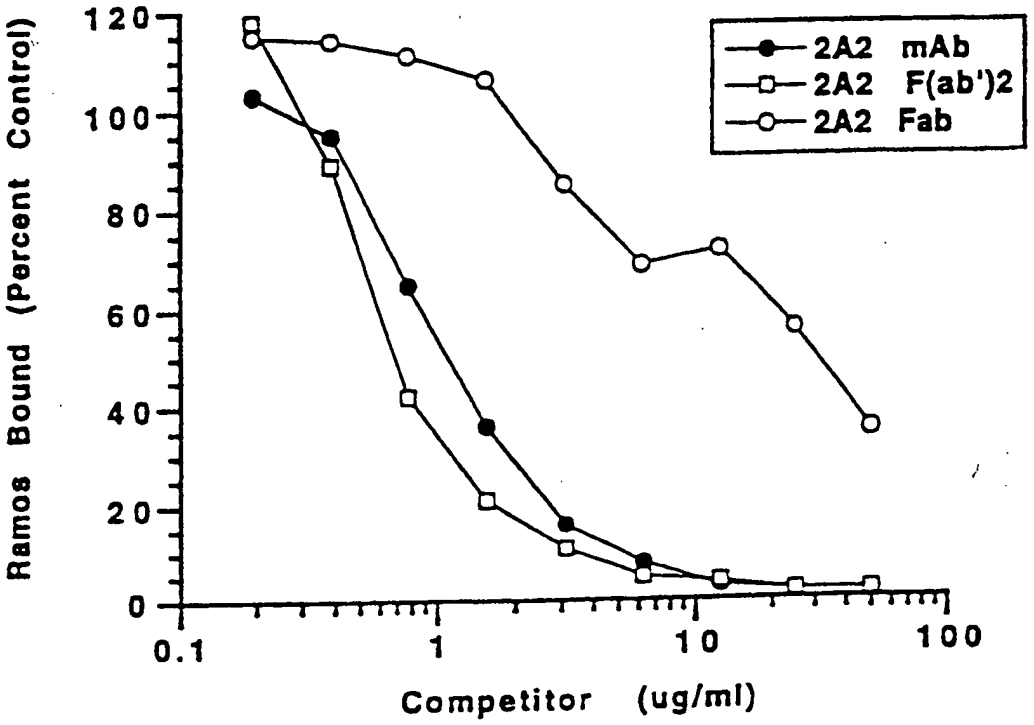
Ramos Bound (Percent Control)
120
100
80
60
40
20
0
0.1
1
10
100
Competitor (ug/ml)
2A2 mAb
2A2 F(ab')2
2A2 Fab

Fig. 8a

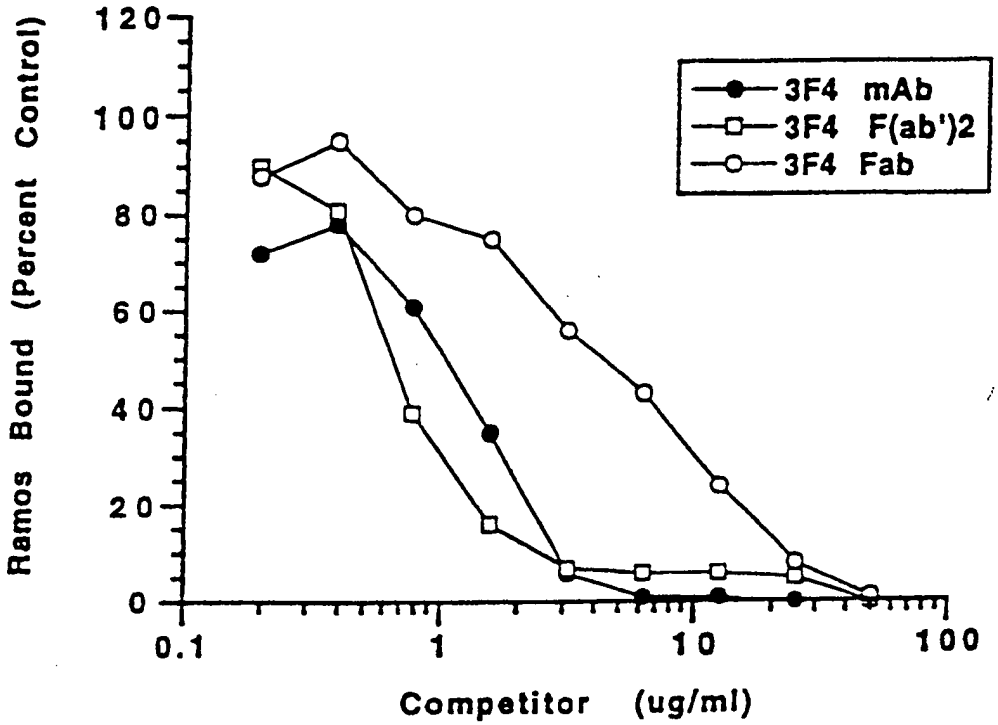
Ramos Bound (Percent Control)
120
100
80
60
40
20
0
0.1
1
10
100
Competitor (ug/ml)
3F4 mAb
3F4 F(ab')2
3F4 Fab

Fig. 8b

VARIABLE LIGHT

```
                                                                  24
D V V M T Q T P L S L P V S L G D Q A S I S C R S 3F4
D I V M T Q S Q K F M S T S L G D R V S V T C K A 2A2

          31 a c d e f   34
S Q S L V H S N G N T Y L Q W Y L Q K P G Q S P K 3F4
S Q N V G P - - - - - N V A W F Q Q K P G Q S P K 2A2

        50          56
L L I Y K V S N R F S G V P D R F S G S G S G T D 3F4
T L I Y S A S F R Y S G V P D R F T G S G S G T D 2A2

                                                  89
F T L K I S R V E A E D L G V Y F C S Q S T H V P 3F4
F T L T I T N V Q S E D L A E Y F C H Q Y N S Y P 2A2

 97
F T F G G G T K L E I K 3F4
L T F G G G T K L K I K 2A2
```

VARIABLE HEAVY

```
Q V Q V Q Q S G A E L A R P W A S V K L S C K A S 3F4
Q V Q L Q Q S G P Q L V R P G T S V K I S C K A S 2A2

          31    35                                        50
G Y N F N S Y W M Q W V K Q R P G Q G L E W I G A 3F4
G Y S F T S Y W M H W V K Q R P G Q D L E W I G M 2A2

 52 a                                 65
I Y P G D G D T S Y T Q K F R G K A T L T A D K S 3F4
I D P S D S E V K L N Q R L K D K A I L T V D K S 2A2

                82 a b c                                  95
S S T A Y M Q L S S L A S E D S A V Y Y C A R R T 3F4
S N T A Y M Q F S G P T S E D S A V Y Y C T R G E 2A2

    100 a 102
V G G Y F D Y W G Q G T T L T V S S 3F4
V S W F - A Y W G Q G T L V T V S A 2A2
```

Fig. 9

SUBSTITUTE SHEET (RULE 26)

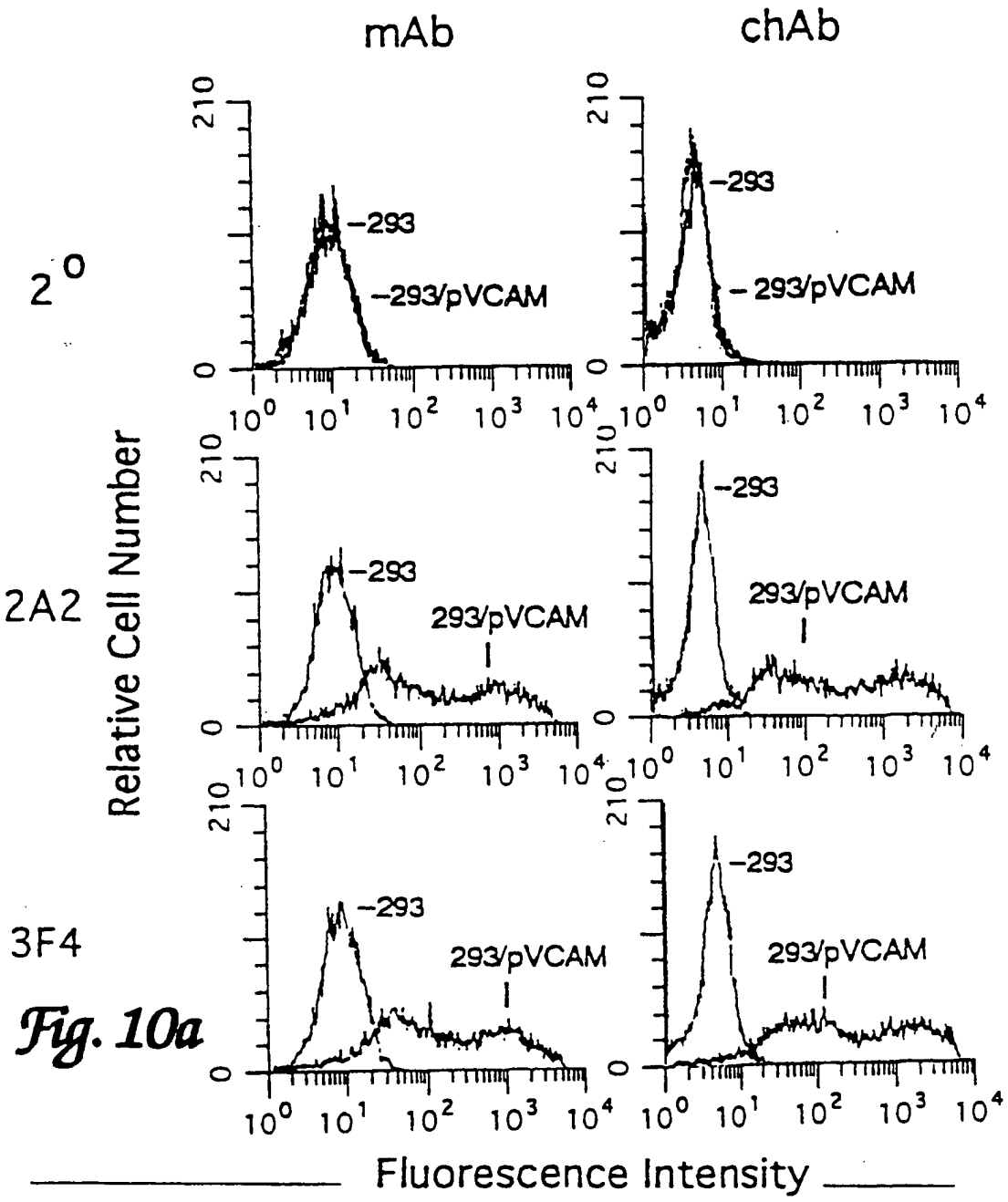

Fig. 10a

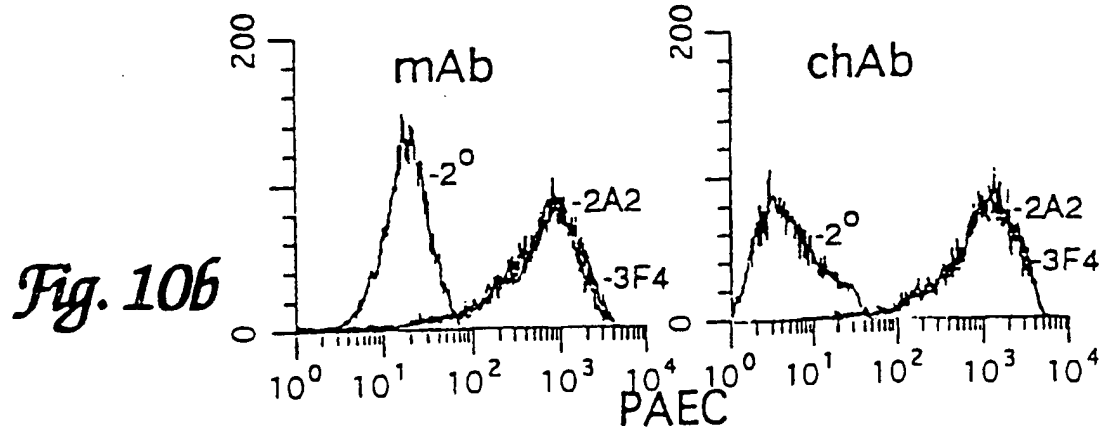

Fig. 10b

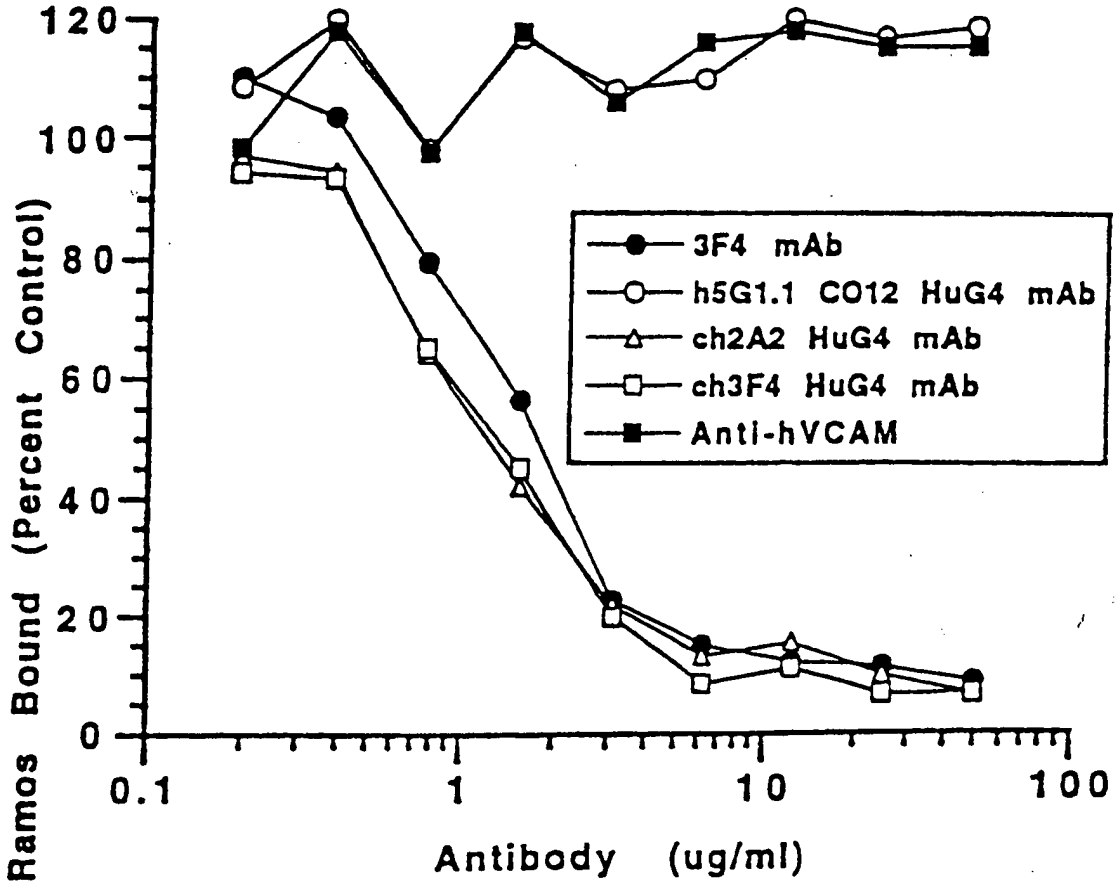
120
100
80
60
40
20
0
Ramos Bound (Percent Control)
0.1
1
10
100
Antibody (ug/ml)
3F4 mAb
h5G1.1 CO12 HuG4 mAb
ch2A2 HuG4 mAb
ch3F4 HuG4 mAb
Anti-hVCAM

Fig. 11

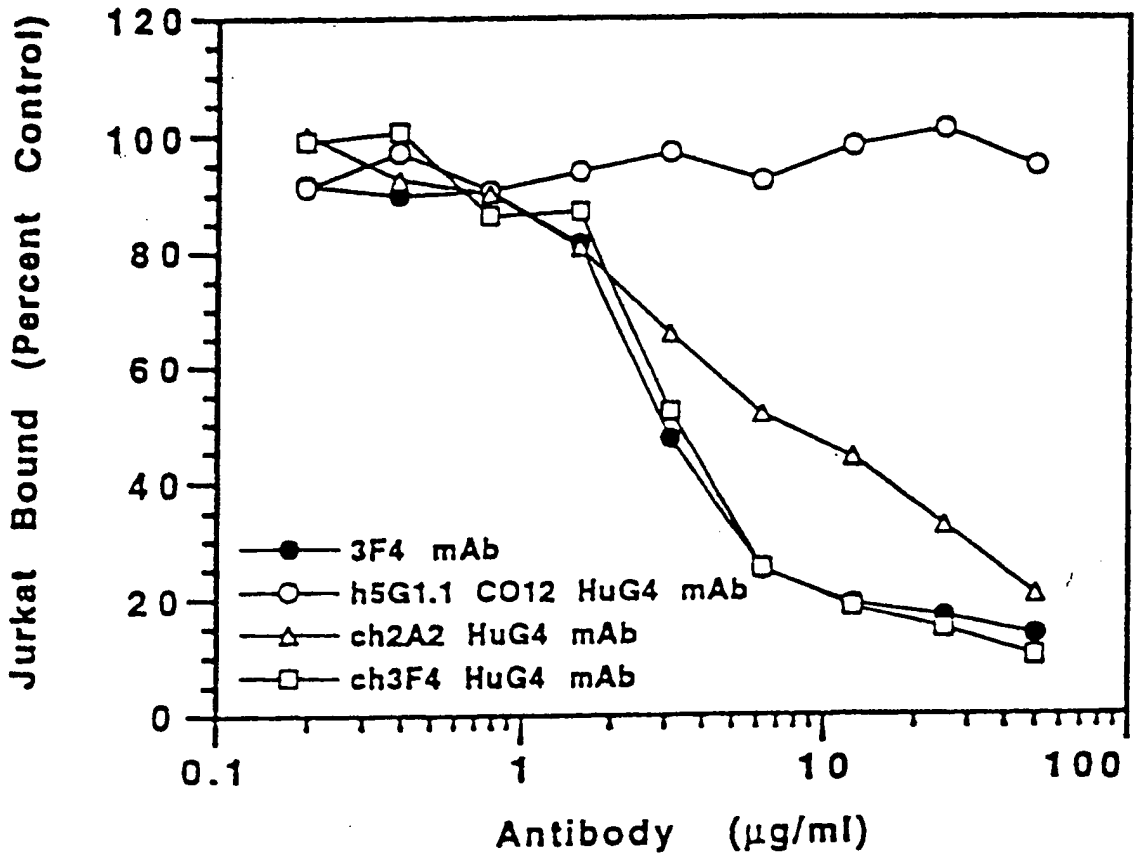
Jurkat Bound (Percent Control)
120
100
80
60
40
20
0
0.1
1
10
100
Antibody (µg/ml)
3F4 mAb
h5G1.1 CO12 HuG4 mAb
ch2A2 HuG4 mAb
ch3F4 HuG4 mAb

Fig. 12

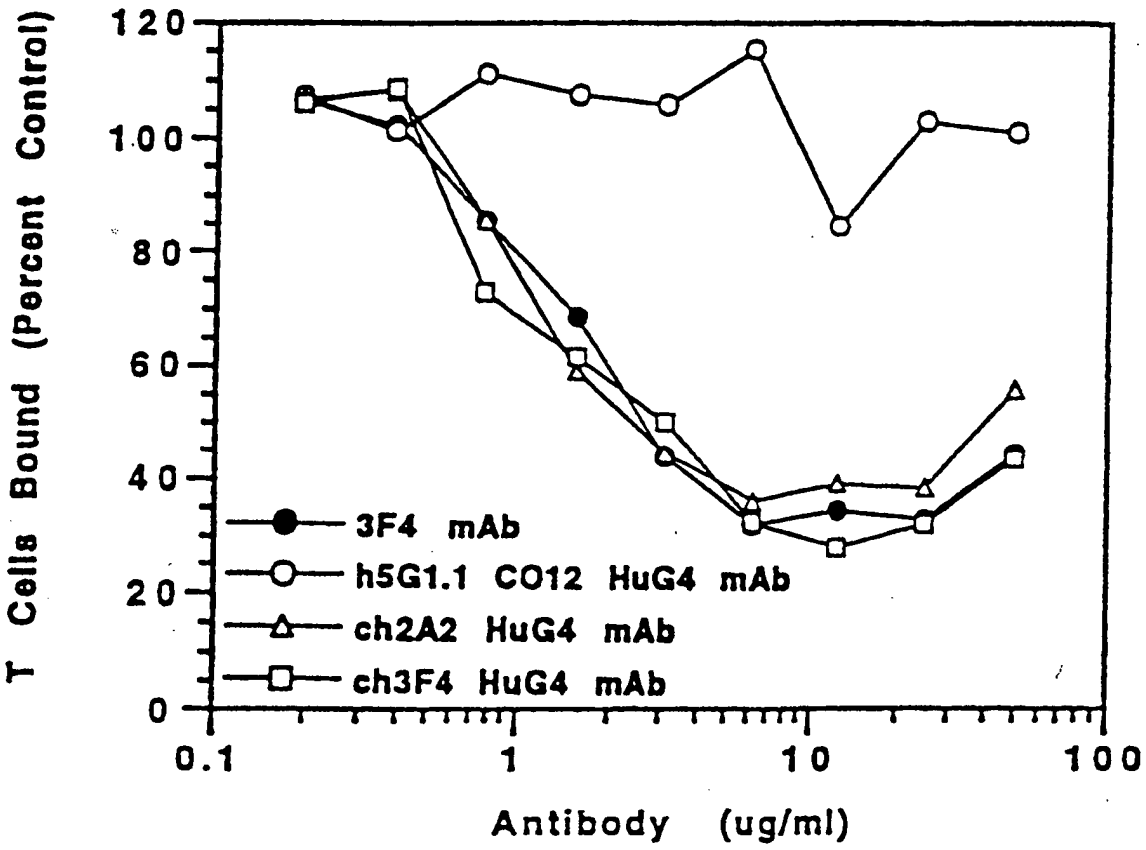
T Cells Bound (Percent Control)
120
100
80
60
40
20
0
0.1
1
10
100
Antibody (ug/ml)
3F4 mAb
h5G1.1 CO12 HuG4 mAb
ch2A2 HuG4 mAb
ch3F4 HuG4 mAb

Fig. 13

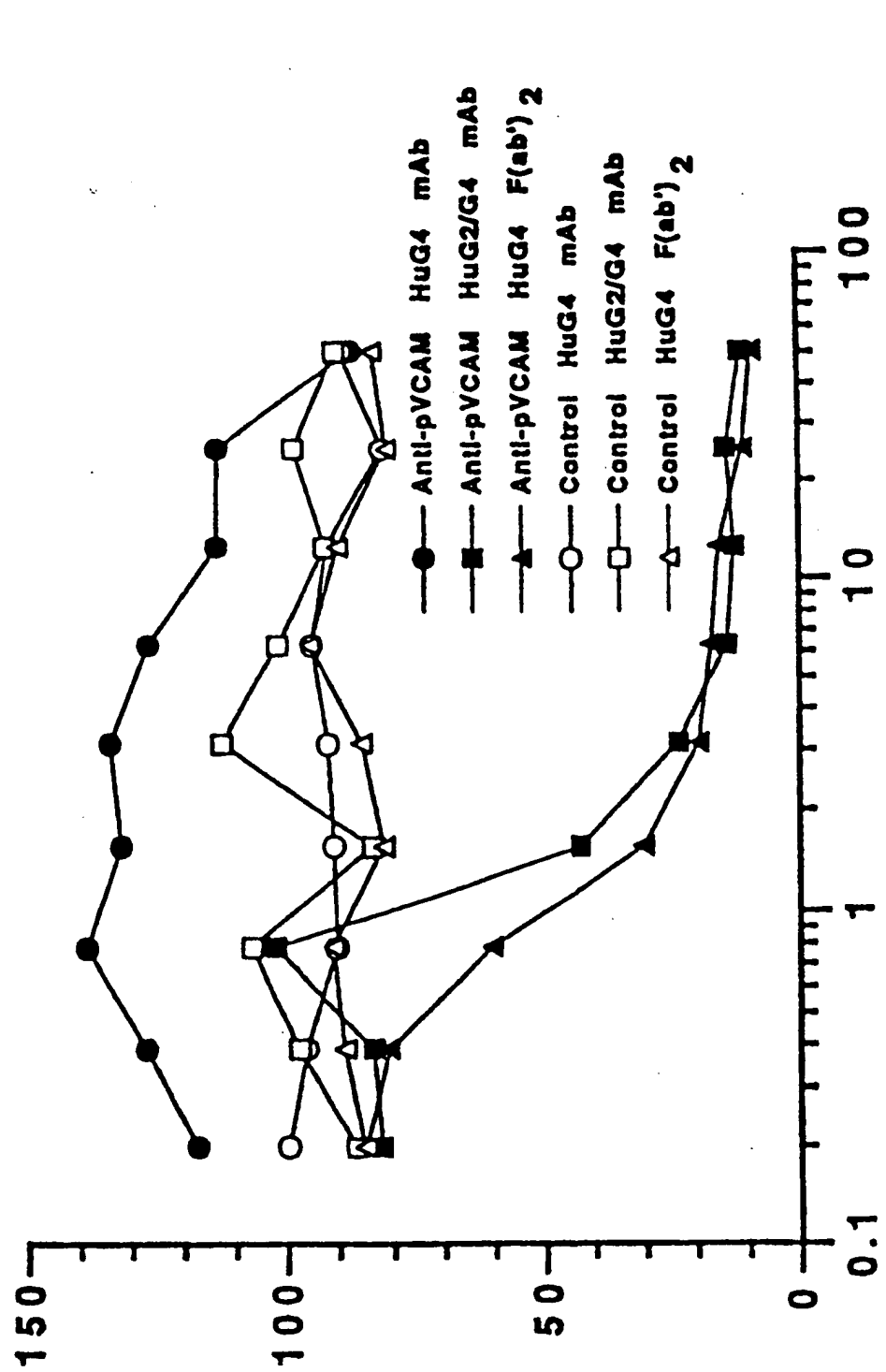
U937 Cells Bound (Percent Control)
150
100
50
0
0.1
1
10
100
Inhibitor (µg/ml)
Anti-pVCAM HuG4 mAb
Anti-pVCAM HuG2/G4 mAb
Anti-pVCAM HuG4 F(ab')2
Control HuG4 mAb
Control HuG2/G4 mAb
Control HuG4 F(ab')2

Fig. 14

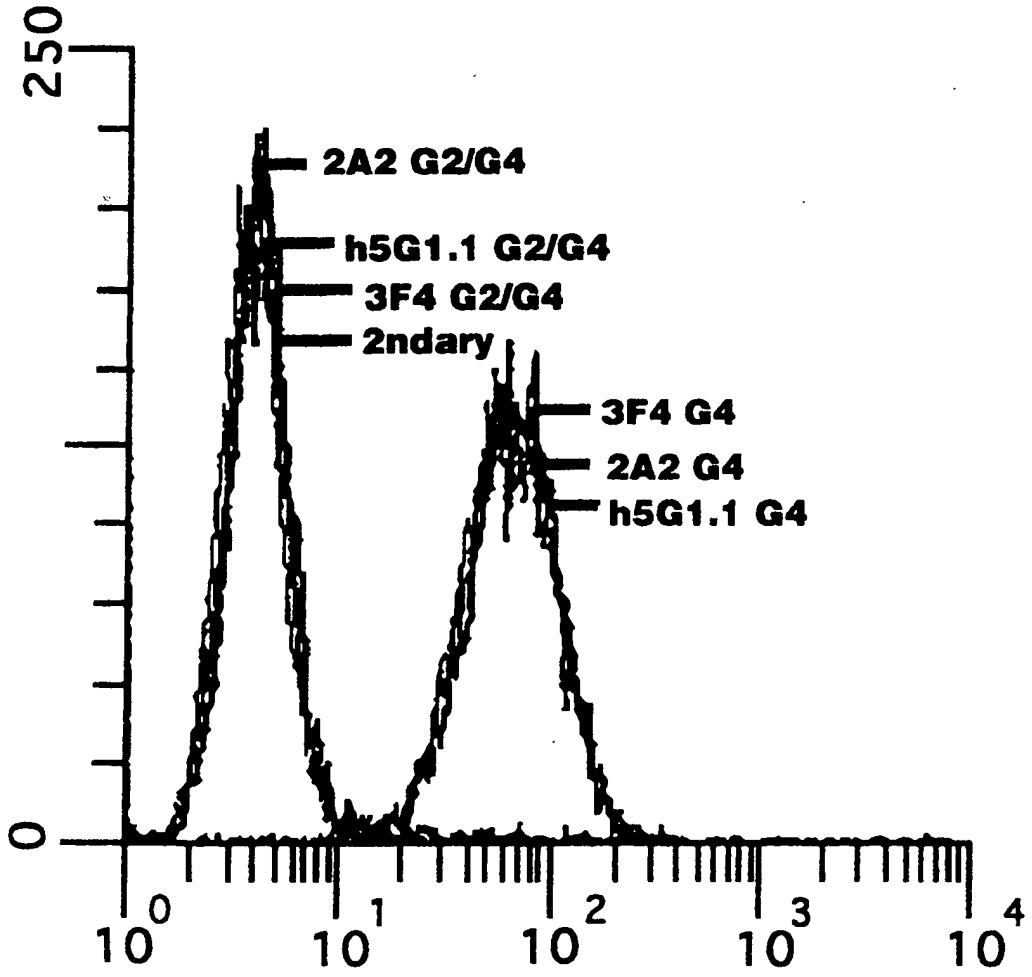
250
0
2A2 G2/G4
h5G1.1 G2/G4
3F4 G2/G4
2ndary
3F4 G4
2A2 G4
h5G1.1 G4
10^0
10^1
10^2
10^3
10^4

Fig. 15

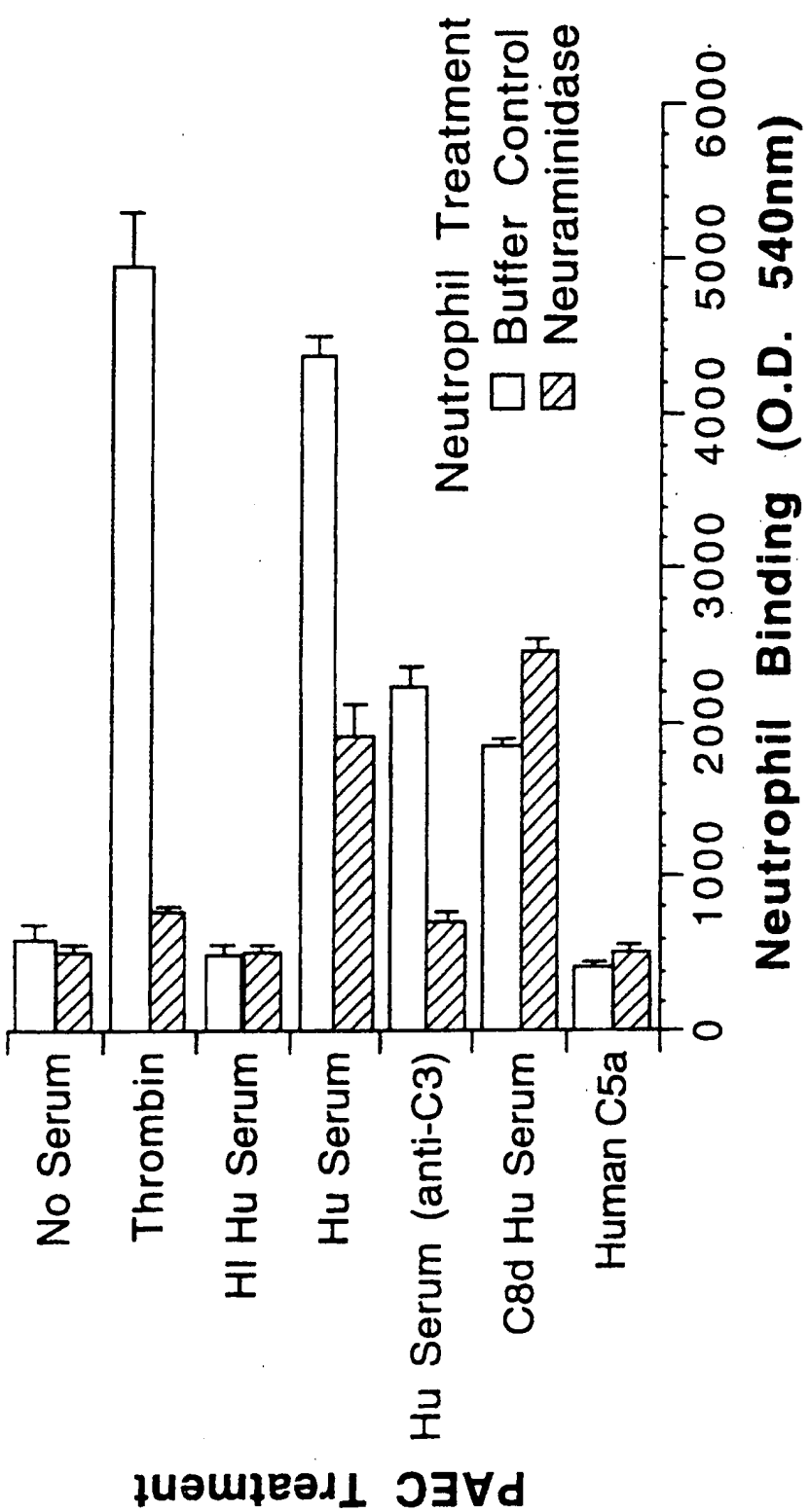
Neutrophil Binding (O.D. 540nm)
0
1000
2000
3000
4000
5000
6000
Neutrophil Treatment
Buffer Control
Neuraminidase
No Serum
Thrombin
HI Hu Serum
Hu Serum
Hu Serum (anti-C3)
C8d Hu Serum
Human C5a
PAEC Treatment

Fig. 16

```
SP  Po MASCLKAICNWRFQRVSFRTVQLLFFNALISDLMNQKGVAA
    Hu **N*QI**LYQ*****V*GIS***C******E*T***E***

Lec Po WTYNYSTSAYSWNTSRVFCQRYFTDLVAIQNKKEIAYLNDVIPYYSSYYWIGMRKINNKWIW
    Hu ***H***K*****I**KY**NRY*********N**D***K*L**********I**N*KT**

    Po VGTKKTLTQEAENWAKNEPNNESNNQDCVEMYIKSPLAPGKWNDEPCVKRKRAL
    Hu *****A**N******D*****KR**E****I*****S********H*L*K*H**

EGF Po CYTASCQSTSCSKQGECTETIGNYTCSCYSGFYGPRCEYVKE
    Hu *******DM********L**********P*****E****R*

CR1 Po CGEFKLPQYVLTNCSHPLGNFSFNSQCSFHCAEGYTLNGPSELECLASGNWTHPPPQCVAVQ
    Hu ***LE***H**M******************TD**QV****K*******I**NK****L*A*

CR2 Po CPALKSPEKGNMACLHSEKAFQYQSSCNFSCEEGYALVGPEVVQCQASGMWTAPVPVCKAIT
    Hu **P**I**R***I****A****H****S******F**********T***V****A*****VQ

CR3 Po Absent
    Hu CQHLEAPSEGTMDCVHPLTAFAYGSSCKFECQPGYRVRGLDMLRCIDSGHWSAPLPTCEAIS

CR4 Po CEPLESPVRGSMDCFPSSRAFQYNTSCSFRCAKGFTLRGADTVRCSNLGQWTAPAPVCQALQ
    Hu ********H*****S**L*****D*N******E**M*****I***D***************

CR5 Po CQDLPAPEKAQVNCSHPFGAFRYQSTCSFTCDEGSSLVGASVLQCLETGNWSAPAPECQ
    Hu *****V*NE*R**************V*****N**LL**********A****NSVP****

    Po GISIVSAPPPEVR
    Hu A*P

CR6 Po Absent
    Hu CTPLLSPQNGTMTCVQPLGSSSYKSTCQFICDEGYSLSGPERLDCTRSGRWTDSPPMCEAIK

CR7 Po Absent
    Hu CPELFAPEQGSLDCSDTRGEFNVGSTCHFSCNNGFKLEGPNNVECTTSGRWSATPPTCK

    Po Absent
    Hu GIASLPTGLQ

CR8 Po CPALITPEQGTMHCQHHLGTFGLNTTCYFRCKTGFTIMGNNALRCRSSGQWTAVAPVCRAVK
    Hu ****T**G****Y*R**P****F******G*NA***LI*DST*SC*P*******T*A*****

CR9 Po CYELHITGPIVMNCSNPWGSFSYGSTCSFHCPEGQLLNGSELTVCKENGEWSTTMPTCL
    Hu *S***VNK**A*****L**N*****I*****L********AQ*A*QE**H****V***Q

    Po AGPLTIQE
    Hu ********

TM  Po ALTYFGGAVASTTGLVMGGTLLALL
    Hu ************I**I*********

CYT Po RKRRRQKDDGKSPLSPQSHLGTYGVFTNAAFDPNP
    Hu ***F*******C**N*H***************S*
```


Fig. 17

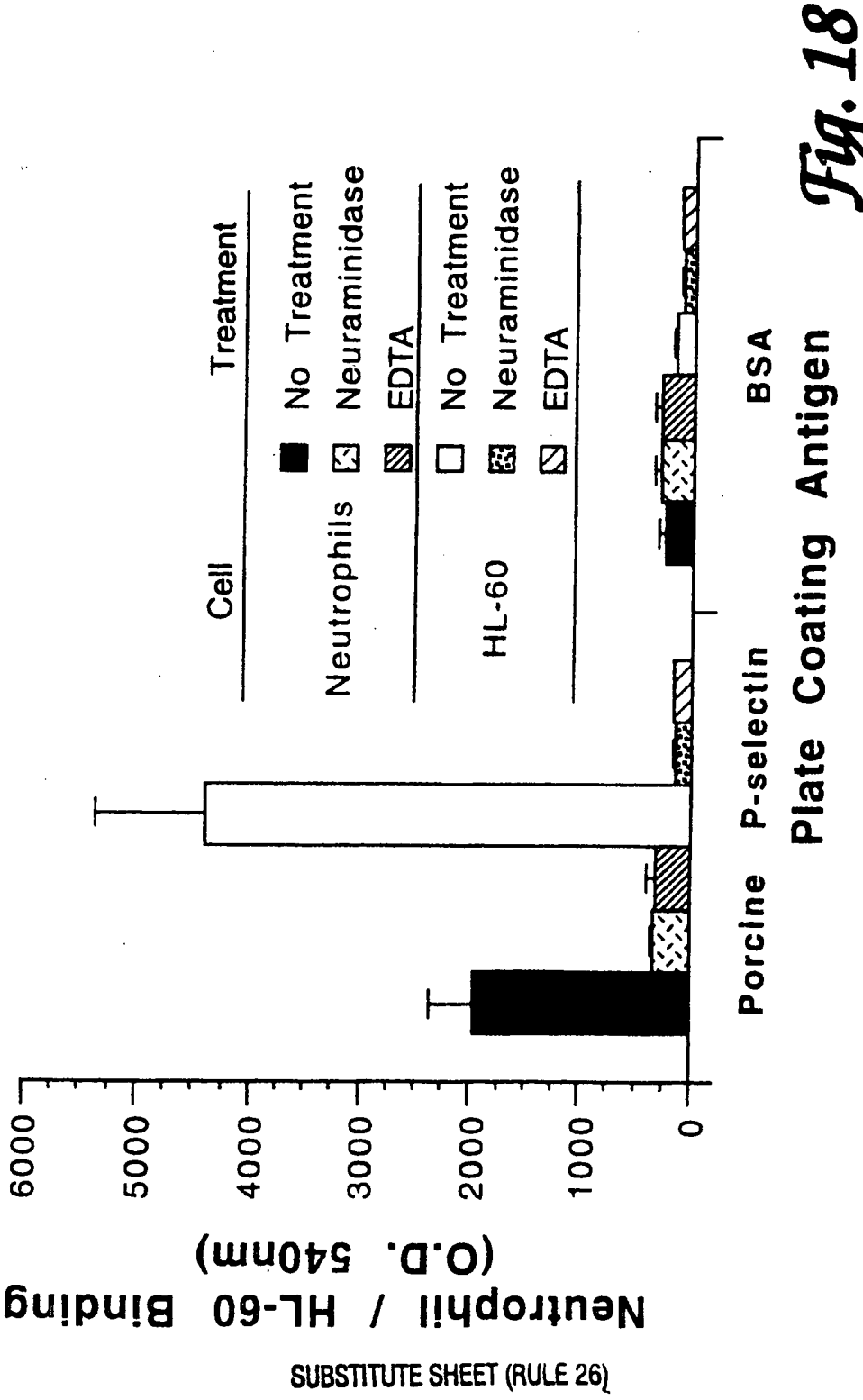
Neutrophil / HL-60 Binding
(O.D. 540nm)
6000
5000
4000
3000
2000
1000
0
Porcine P-selectin
BSA
Plate Coating Antigen
Cell
Treatment
Neutrophils
No Treatment
Neuraminidase
EDTA
HL-60
No Treatment
Neuraminidase
EDTA

Fig. 18

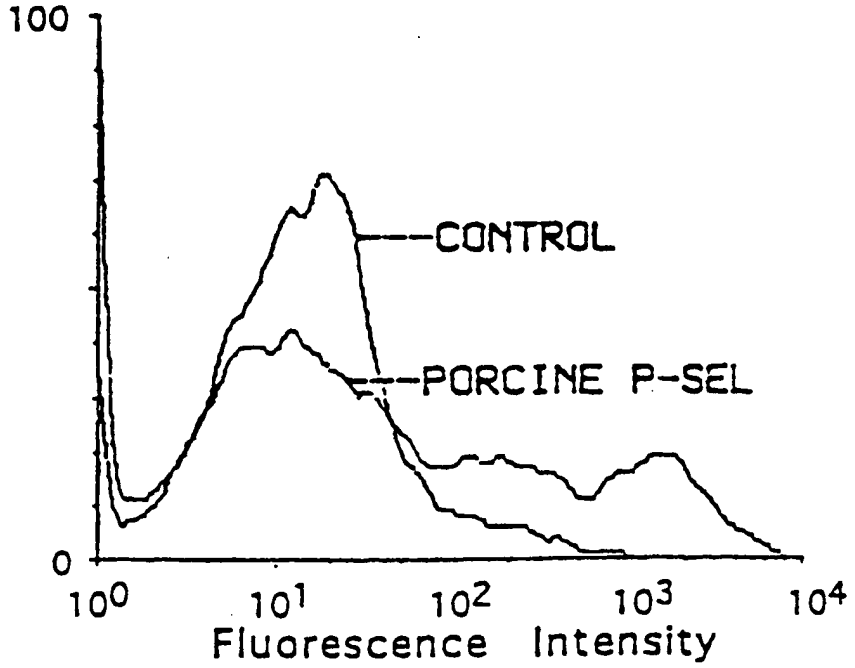

Fig 19a

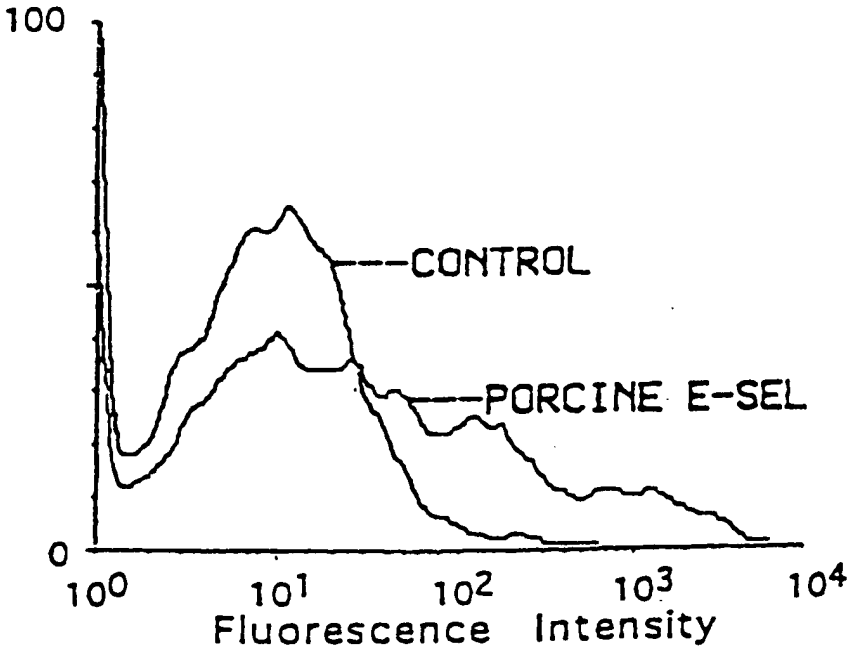

Fig. 19b

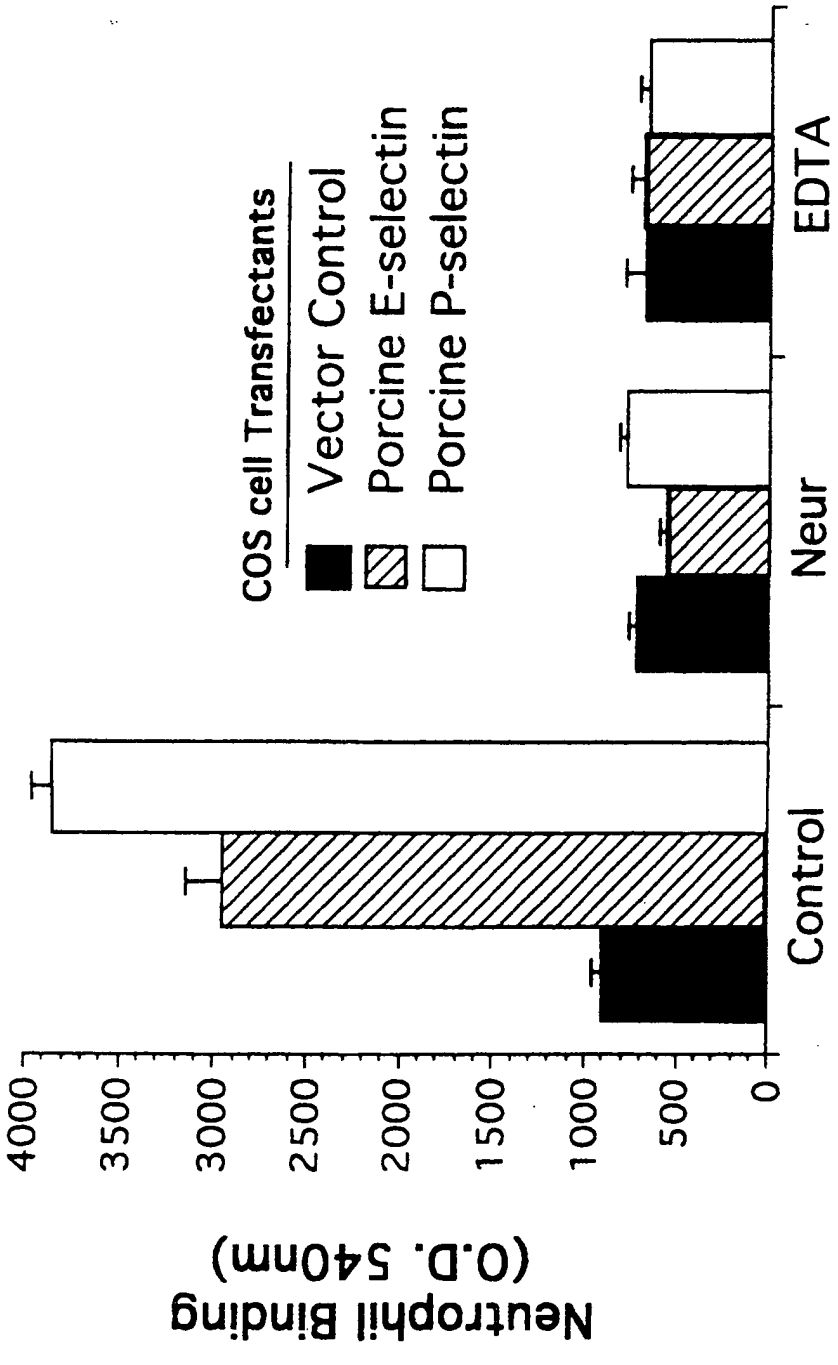
Neutrophil Binding
(O.D. 540nm)
4000
3500
3000
2500
2000
1500
1000
500
0
Control
Neur
EDTA
COS cell Transfectants
Vector Control
Porcine E-selectin
Porcine P-selectin

Fig. 20

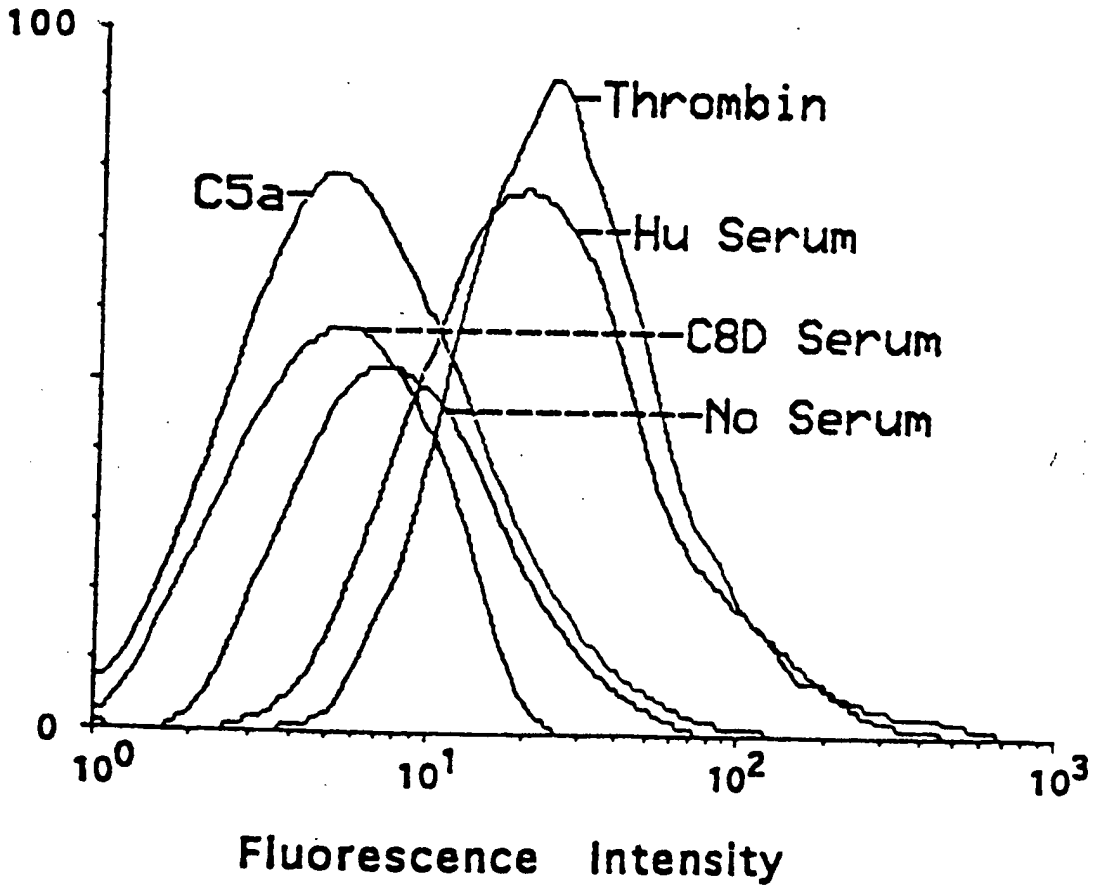
100
0
Thrombin
C5a
Hu Serum
C8D Serum
No Serum
10^0
10^1
10^2
10^3
Fluorescence Intensity

Fig. 21

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US96/15575

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :C07K 16/00, 16/18, 16/28, 16/46
US CL : 530/387.1, 387.2, 388.1, 388.22, 388.7, 388.73
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : C07K 16/00, 16/18, 16/28, 16/46

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

APS, DIALOG, BIOSIS, CA, EMBASE, MEDLINE, WPI
search terms: porcine, swine, pig, vcam, cd86, p selectin

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	Biochem. Biophys. Res. Commun., Volume 201, Number 2, issued 15 June 1994, Tsang et al., "Cloning and Expression Kinetics of Porcine Vascular Cell Adhesion Molecule", pages 805-812, see entire document.	1-4
X	Xenotransplantation, Volume 2, issued 1995, Kumagai-	1-4
---	Braesch et al., "Identification of Swine and Primate Cellular	--------
Y	Adhesion Molecules (CAM) Using Mouse Anti-Human Monoclonal Antibodies", pages 88-97, see entire document.	1-4, 8

☐ Further documents are listed in the continuation of Box C. ☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
05 DECEMBER 1996	15 JAN 1997
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer PHILLIP GAMBEL Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

[redacted]ational application No.
PCT/US96/15575

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 5-7
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

SEQUENCE SEARCH COULD NOT BE PERFORMED BECAUSE OF DEFECTIVE DISKETTE SUBMISSION.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.